

Katcheves, Konstantina

From: Katcheves, Konstantina
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Subject: Application serial number 09/433429

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Experimental testicular teratoma promotes formation of humor immune response in th host testis
Sundstrom et al.
Journal of Reproductive immunology
Vol. 42 no.2
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Thank you,
Tina

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(FILE 'HOME' ENTERED AT 09:34:17 ON 24 JUL 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS' ENTERED AT 09:34:28 ON 24 JUL 2003

L1 373 S IMMUNOLOGICALLY PRIVILEGED SITE

L2 214 DUP REMOVE L1 (159 DUPLICATES REMOVED)

L3 10 S L2 AND SERTOLI CELLS

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Pagination. e-file.
ISSN: 0098-1133.

DOCUMENT TYPE:

Patent

LANGUAGE:

English

AB A method of treating a disease is provided that results from a deficiency of a biological factor which comprises administering to a mammal **Sertoli cells** and also cells that produce the biological factor. A method of treating diabetes mellitus is carried out by transplanting pancreatic islet of Langerhans cells in conjunction with **Sertoli cells** to create an **immunologically privileged site**. A method of creating an **immunologically privileged site** and providing cell stimulatory factors in a mammal for transplants is also carried out. A method of co-localizing islet cells with **Sertoli cells** and the use of the co-localized product for treating diabetes mellitus is further provided. Further described is a method of creating systemic tolerance to foreign antigens. A method of enhancing the viability, maturation, proliferation of functional capacity of cells in tissue culture is also provided. In addition, pharmaceutical compositions comprising **Sertoli cells** and cells that produce a biological factor, and/or a pharmaceutically acceptable carrier are further provided.

L3 ANSWER 9 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN
 ACCESSION NUMBER: 1997:127489 CAPLUS
 DOCUMENT NUMBER: 126:135688
 TITLE: Use of co-localized islets and **Sertoli cells** in xenograft cellular transplants
 INVENTOR(S): Selawry, Helena P.
 PATENT ASSIGNEE(S): Research Corporation Technologies, Inc., USA
 SOURCE: PCT Int. Appl., 109 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9640178	A1	19961219	WO 1996-US9627	19960607
W: AU, CA, JP, MX, NO RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
CA 2187803	AA	19951026	CA 1995-2187803	19950413
JP 09512015	T2	19971202	JP 1995-527083	19950413
US 5725854	A	19980310	US 1995-421641	19950413
US 5759534	A	19980602	US 1995-467338	19950606
US 5843430	A	19981201	US 1995-467341	19950606
US 5849285	A	19981215	US 1995-485340	19950607
AU 9661056	A1	19961230	AU 1996-61056	19960607
US 5958404	A	19990928	US 1996-660258	19960607
FI 9604033	A	19961212	FI 1996-4033	19961008
US 6149907	A	20001121	US 1999-298015	19990423
PRIORITY APPLN. INFO.:			US 1995-485340	A 19950607
			US 1994-211695	A 19940413
			US 1995-421641	A1 19950413
			WO 1995-US4565	W 19950413
			US 1996-660258	A3 19960607
			WO 1996-US9627	W 19960607

AB The present invention describes a method of treating a disease that results from a deficiency of a biol. factor which comprises administering to a mammal **Sertoli cells** and cells that produce the biol. factor. In particular, the present invention describes a method of treating diabetes mellitus by transplanting pancreatic islet of Langerhans cells in conjunction with **Sertoli cells** to create an immunol. privilege site. A method of creating an **immunol. privileged site** and providing cell stimulatory factors in a mammal for transplants further described by the present invention. A method of co-localizing islet cells with **Sertoli cells** and the use of the co-localized product treating diabetes mellitus is further provided. The present invention further describes a method of creating systemic tolerance to foreign antigens. A method of enhancing the viability, maturation, proliferation of functional capacity of cells in tissue culture is further provided. A pharmaceutical compn. comprising **Sertoli cells** and cells that produce a biol. factor is also provided.

L3 ANSWER 10 OF 10 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 ACCESSION NUMBER: 2001:260992 BIOSIS
 DOCUMENT NUMBER: PREV200100260992
 TITLE: Treatments using **sertoli cells**.
 AUTHOR(S): Selawry, Helena P. (1)
 CORPORATE SOURCE: (1) Rileyville, VA USA
 ASSIGNEE: Research Corporation Technologies, Inc.
 PATENT INFORMATION: US 6149907 November 21, 2000
 SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Nov. 21, 2000) Vol. 1240, No. 3, pp. No

L3 ANSWER 7 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2002:409243 CAPLUS

DOCUMENT NUMBER: 136:395972

TITLE: Methods of treating disease using **Sertoli** cells and allografts or xenografts

INVENTOR(S): Selawry, Helena P.; Cameron, Don Frank

PATENT ASSIGNEE(S): USA

SOURCE: U.S. Pat. Appl. Publ., 26 pp.

CODEN: USXXCO

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002065212	A1	20020530	US 1996-747122	19961108
-----			US 1996-747122	19961108

PRIORITY APPLN. INFO.: US 1996-747122 19961108

AB The invention describes a method for the treatment of a disease that results from a deficiency of a biol. factor which comprises administration of **Sertoli** cells and cells that produce the biol. factor to a mammal. In particular, the invention describes a method for the treatment of diabetes mellitus by transplanting pancreatic islet of Langerhans cells in conjunction with **Sertoli** cells to create an **immunol. privileged site**. A method for creating an **immunol. privileged site** and providing cell stimulatory factors in a mammal for transplants is further described by the invention. The invention further describes a method for creating systemic tolerance to foreign antigens. A method for enhancing the viability, maturation, proliferation of functional capacity of cells in tissue culture is further provided. A pharmaceutical compn. comprising **Sertoli** cells and cells that produce a biol. factor is also provided. In addn., treatment of an autoimmune disease via the transplantation of **Sertoli** cells alone into a transplant site other than the testes is disclosed. The dosage amt. of **Sertoli** cells administered ranges from 105 to 1010 cells. Also, an in vitro method for accelerating the maturation and increasing the proliferation and functional capacity of proliferating mammalian cells via the co-culturing of the mammalian cells with **Sertoli** cells is disclosed.

L3 ANSWER 5 OF 10 MEDLINE on STN
ACCESSION NUMBER: 85293354 MEDLINE
DOCUMENT NUMBER: 85293354 PubMed ID: 2863395
TITLE: The role of germinal epithelium and spermatogenesis in the
privileged survival of intratesticular grafts.
AUTHOR: Whitmore W F 3rd; Karsh L; Gittes R F
CONTRACT NUMBER: R01 AI 18138 (NIAID)
SOURCE: JOURNAL OF UROLOGY, (1985 Oct) 134 (4) 782-6.
Journal code: 0376374. ISSN: 0022-5347.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 198510
ENTRY DATE: Entered STN: 19900320
Last Updated on STN: 19970203
Entered Medline: 19851021

AB The testis is an **immunologically privileged site**. Since earlier studies excluded testicular steroid production as an essential factor, the present study evaluates the role of germ cells and spermatogenesis in the privileged survival of allografts within the testis. We used a Sertoli cell-only testis model and adolescent unilateral cryptorchidism in inbred rats to eliminate germ cells and spermatogenesis selectively. Parathyroid allografts were implanted into these sites, normal testes and beneath the renal capsule (a nonprivileged site) in appropriately matched controls. With at least 15 rats in each group, privileged allograft survival was shown to be unaffected by eliminating germ cells and spermatogenesis (p less than .005). Experimental evidence suggests the presence of an active process which incidentally permits privileged allograft survival within the testis, but which exists teleologically to protect the developing sperm from autoimmune attack. This is in addition to the passive anatomical separation provided by the blood-testis barrier. Our cumulative data strongly implicates the Sertoli cell in this process.

L3 ANSWER 3 OF 10 MEDLINE on STN
ACCESSION NUMBER: 94191899 MEDLINE
DOCUMENT NUMBER: 94191899 PubMed ID: 8143079
TITLE: Sertoli cell-enriched fractions in successful islet cell transplantation.
AUTHOR: Selawry H P; Cameron D F
CORPORATE SOURCE: Department of Veterans Affairs Medical Center, Memphis, TN 38104.
SOURCE: CELL TRANSPLANTATION, (1993 Mar-Apr) 2 (2) 123-9.
Journal code: 9208854. ISSN: 0963-6897.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199405
ENTRY DATE: Entered STN: 19940511
Last Updated on STN: 19940511
Entered Medline: 19940505

AB Prolonged survival of Islet- allo- and xenografts can be induced following implantation of the islets into the abdominal testis of diabetic rats. We previously showed that a factor released by **Sertoli cells** appears to be responsible for the protection of the intratesticular islet allo- and xenografts against rejection. The aim of this study was to examine whether an **immunologically privileged site** can be established in an organ site *in vivo*, other than the testis, such as the renal, subcapsular space, to make feasible the grafting of female recipients as well. A total of 36 male and 21 female, diabetic, PVG rats were divided into six different treatment groups: 1) Six male rats were grafted with islets from Sprague-Dawley (S-D) donor rats only. 2) Ten male rats were grafted with islets from (S-D) donors and were then given a short course of cyclosporine (CsA) posttransplantation. 3) Ten male rats were grafted with islets from (S-D) donors and with Sertoli cell-enriched fractions (SEF) from PVG donors but without CsA. 4) Ten male rats were grafted with a combination of islets from (S-D) and SEF from (PVG), donors, respectively, and CsA. 5) Ten female rats were given an identical combination of cells and CsA as depicted for group 5. 6) Ten female rats were grafted with a combination of islets and SEF, both cell types from S-D donors, and CsA. The results showed that 70% to 100% of the grafted rats in groups 1, 2, and 3 remained hyperglycemic. (ABSTRACT TRUNCATED AT 250 WORDS)

L3 ANSWER 6 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:964902 CAPLUS
DOCUMENT NUMBER: 138:20500
TITLE: Production of a biological factor and creation of an immunologically privileged environment using genetically altered **Sertoli cells**
INVENTOR(S): Kirkpatrick, Shaun A.; Gores, Paul; Halberstadt, Craig
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 10 pp., Division of U.S. Ser. No. 433,429.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002192200	A1	20021219	US 2002-219804	20020815
PRIORITY APPLN. INFO.:			US 1999-433429	A3 19991104

AB The present invention provides a method of providing an individual with a biol. factor or intermediate thereof which comprises introducing into the individual **Sertoli cells** genetically altered to produce the biol. factor or intermediate thereof. The genetically altered **Sertoli cells** are administered in an amt. effective to produce the desired effect. Aside from producing the biol. factor or intermediate thereof, the engineered **Sertoli cells** also create an **immunol. privileged site**. Vectors comprising a promoter which functions in **Sertoli cells** operably linked to coding sequence for a desired biol. factor are also provided as are **Sertoli cells** comprising such vectors. A pharmaceutical compn. comprising **Sertoli cells** genetically altered to produce a biol. factor is also provided.

(FILE 'HOME' ENTERED AT 14:33:01 ON 24 JUL 2003)

FILE 'MEDLINE, BIOSIS, CAPLUS, EMBASE' ENTERED AT 14:33:14 ON 24 JUL 2003

L1 817 S HEMOPHILIA AND DOG
L2 512 DUP REMOVE L1 (305 DUPLICATES REMOVED)
L3 172 S L2 AND FACTOR IX
L4 100 S L3 AND (VECTOR OR GENE THERAPY)
L5 2 S L4 AND IMMUNOLOGICAL RESPONSE
L6 65 S L4 AND EXPRESSION
L7 8 S L6 AND (PROBLEM OR DIFFICULTIES OR CHALLENGES OR CHALLENGE O

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(FILE 'HOME' ENTERED AT 14:33:01 ON 24 JUL 2003)

FILE 'MEDLINE, BIOSIS, CAPLUS, EMBASE' ENTERED AT 14:33:14 ON 24 JUL 2003

L1 817 S HEMOPHILIA AND DOG
L2 512 DUP REMOVE L1 (305 DUPLICATES REMOVED)
L3 172 S L2 AND FACTOR IX
L4 100 S L3 AND (VECTOR OR GENE THERAPY)
L5 2 S L4 AND IMMUNOLOGICAL RESPONSE
L6 65 S L4 AND EXPRESSION
L7 8 S L6 AND (PROBLEM OR DIFFICULTIES OR CHALLENGES OR CHALLENGE O
L8 619 S IMMUNOLOGICALLY PRIVILEGED SITE OR IMMUNOPRIVILEGED SITE
L9 30 S L8 AND PANCREAS
L10 20 DUP REMOVE L9 (10 DUPLICATES REMOVED)

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(FILE 'HOME' ENTERED AT 15:23:59 ON 24 JUL 2003)

FILE 'CAPLUS, BIOSIS, EMBASE' ENTERED AT 15:24:09 ON 24 JUL 2003

L1 218 S CELL REPLACEMENT THERAPY
L2 32 S L1 AND GENE EXPRESSION
L3 26 DUP REMOVE L2 (6 DUPLICATES REMOVED)
L4 0 S L3 AND (PROBLEMS OR CHALLENGES OR OBSTACLES OR DIFFICULTIES)
L5 3675 S EX VIVO AND GENE AND EXPRESSION
L6 58 S EX VIVO GENE DELIVERY
L7 36 DUP REMOVE L6 (22 DUPLICATES REMOVED)
L8 521 S EX VIVO GENE THERAPY
L9 33 S L8 AND (PROBLEMS OR CHALLENGES OR DIFFICULTIES OR OBSTACLES)

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L3 ANSWER 2 OF 10 MEDLINE on STN
ACCESSION NUMBER: 1999236923 MEDLINE
DOCUMENT NUMBER: 99236923 PubMed ID: 10221734
TITLE: Experimental testicular teratoma promotes formation of humoral immune responses in the host testis.
AUTHOR: Sundstrom J; Verajnkorva E; Salminen E; Pelliniemi L J; Pollanen P
CORPORATE SOURCE: Department of Anatomy, University of Turku, Finland..
Jari.Sundstrom@utu.fi
SOURCE: JOURNAL OF REPRODUCTIVE IMMUNOLOGY, (1999 Mar) 42 (2) 107-26.
Journal code: 8001906. ISSN: 0165-0378.
PUB. COUNTRY: Ireland
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199906
ENTRY DATE: Entered STN: 19990714
Last Updated on STN: 19990714
Entered Medline: 19990629

AB The testis is an **immunologically privileged site**. Very little is known about the factors regulating formation of immune responses elicited by a neoplasm in the testis. We have studied the immune response of the host testis against experimental testicular teratoma in mouse by localizing adhesion molecules (CD106, CD54, CD49d/CD29, CD44, CD18, CD8 and CD4), cytokines (IL-2, IL-4, IL-6, IL-10 and IL-12), T-cell costimulators (CD80, CD86) and the lipid antigen presenting molecule CD1d in the testis of 129/SvJ mice with and without experimental testicular teratoma. The testicular teratomas were induced by grafting male gonadal ridges from 12-day-old 129/SvJ mouse fetuses into testes of adult mice from the same strain. The tumors cultured intratesticularly for 2, 3, 4 and 8 weeks (three animals per time point) were used for immunocytochemistry. CD1d was detected in **Sertoli cells** and in some degenerated tubules of the host testis surrounding the graft. In the tumor, CD1d was detected in glandular epithelia, smooth muscle and in thin fibers of neural origin. IL-2 was observed in some blood vessels of the host testis and of the tumor and in occasional cell infiltrates around these vessels. Some tubular structures of the tumor were also positive for IL-2. IL-6 was detected in **Sertoli cells** of the normal testis and in **Sertoli cells** and in solitary interstitial cells as well as in the walls of some blood vessels of the host testis. The reaction for IL-6 was more prominent in the tubules apparently damaged by the growing tumor. In the tumor IL-6 was detected in epithelial structures, muscle cells, in thin fibers of neural origin and in some blood vessels. IL-10 was detected in individual cells in the interstitium and in degenerating tubules of the host testis. In the tumor the epithelial structures were positive for IL-10. The interstitium of the host testis was positive for CD106 and the embryonic testicular cords in the graft were also positive, but the tumor was negative. CD44 and CD18 were observed in some blood vessels and in degenerated tubules of the host testis. In the tumor CD44 and CD18 were occasionally observed in cartilage and in epithelial structures. The results of the present study suggest that cytokine microenvironment in the testis containing neoplastic tissue promotes activation of humoral immune responses. In addition, as the damaged seminiferous tubules expressed increased amounts of two cytokines promoting humoral immune responses, IL-6 and IL-10, it is possible that also in other conditions with damage to the tubules, humoral immune responses predominate.

L7 ANSWER 4 OF 8 MEDLINE on STN
ACCESSION NUMBER: 1998447105 MEDLINE
DOCUMENT NUMBER: 98447105 PubMed ID: 9776210
TITLE: **Problems and prospects in gene therapy for hemophilia.**
AUTHOR: Herzog R W; High K A
CORPORATE SOURCE: The Children's Hospital of Philadelphia, Abramsom Research Center, PA 19104, USA.
SOURCE: CURRENT OPINION IN HEMATOLOGY, (1998 Sep) 5 (5) 321-6.
Ref: 49
Journal code: 9430802. ISSN: 1065-6251.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199812
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 19990115
Entered Medline: 19981211

AB The aim of **gene therapy** for **hemophilia** is the stable introduction and **expression** of a gene encoding functional blood coagulation factor VIII or IX. Although there are as yet no published studies demonstrating long-term **expression** of therapeutic levels in large animal models of the disease, there have been several reports over the past year of sustained **expression** of therapeutic levels of clotting factors in small animals, and some of these strategies are currently being applied to hemophilic **dogs**. Recent advances include optimized retroviral gene transfer, improved adenoviral **vectors** for high levels of sustained **expression** of factor VIII in mice, stable therapeutic levels of **factor IX expression** in mice after transduction of muscle or liver with adenoassociated virus **vector**, as well as new nonviral gene delivery strategies. Finally, several important mouse and **dog** models of **hemophilia** have been characterized during the past year.

L7 ANSWER 6 OF 8 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2003:328494 BIOSIS
DOCUMENT NUMBER: PREV200300328494
TITLE: AAV-mediated gene transfer for **hemophilia**:
Problems and prospects.
AUTHOR(S): High, Katherine A. (1)
CORPORATE SOURCE: (1) Children's Hosp. of Philadelphia, Philadelphia, PA, USA
USA
SOURCE: Abstracts of the Interscience Conference on Antimicrobial
Agents and Chemotherapy, (2002) Vol. 42, pp. 468-469.
print.
Meeting Info.: 42nd Interscience Conference on
Antimicrobial Agents and Chemotherapy San Diego, CA, USA
September 27-30, 2002 American Society for Microbiology

DOCUMENT TYPE: Conference
LANGUAGE: English

AB Our research efforts have been focussed on developing a gene transfer strategy for the treatment of the **hemophilias**.

Hemophilia is an attractive target for studies in gene transfer because even small amounts of clotting factor can improve the clinical symptoms of the disease, the factor can be expressed in almost any tissue as long as it gains access to the circulation, and there are large and small animal models of the disease, so that promising approaches can be assessed for efficacy before moving into clinical studies (High, Circulation Research, 2001). We have developed recombinant adeno-associated viral (AAV) **vectors** expressing blood coagulation **Factor IX**. AAV has a number of advantages as a gene transfer **vector** including: 1) the absence of viral coding sequences in the recombinant **vector**; 2) the ability to transduce a variety of non-dividing target cells, including liver, muscle and nervous system; 3) the ability to direct long-term **expression** of the transgene in immunocompetent animals. We have introduced AAV-F.IX **vectors** into skeletal muscle and liver, and shown long-term correction of the bleeding diatheses in both small and large animal models of **hemophilia** B (Herzog et al., PNAS, 1997; Nakai et al., Blood, 1998; Herzog et al., Nature Medicine, 1999; Herzog et al., Blood, 2002). In the initial clinical trial, rAAV was introduced into skeletal muscle of subjects with severe **hemophilia** B. Results showed that the general characteristics of transduction were similar in mouse, canine and human muscle, and muscle biopsies of injected sites showed evidence of gene transfer and **expression**, but circulating levels of F.IX were generally less than 1%, failing to reach the desired target of 3-10%. There were no serious adverse events associated with rAAV injection in skeletal muscle (Kay et al., Nature Genetics, 2000). More recent work has focussed on an intravascular route of delivery to skeletal muscle that has resulted in circulating levels of F.IX of apprx10% in **hemophilia** B **dogs**. Work has also proceeded on development of a liver-directed approach. Engineering of the **expression** cassette has resulted in better **expression** per particle, and circulating F.IX levels of 4-12% have now been achieved in **hemophilia** B **dogs** treated with **vector** doses lower than those already administered in the clinical study in skeletal muscle (Herzog et al., Blood 99:2670, 2002). After extensive safety studies in mice, rats, hemophilic **dogs** and non-human primates, a Phase I study of an AAV-mediated, liver-directed approach to treating **hemophilia** B has begun. There were no acute toxicities associated with administration of **vector** to the first two subjects, but subsequently a PCR assay on the subjects' semen was found to be positive for **vector** sequences. After a period of weeks, the positive signal disappeared. These findings were distinct from those seen in pre-clinical animal studies. To gain a clearer understanding of the biodistribution of **vector** to the gonads, we undertook additional studies in rabbits and mice. These

showed that, following intravascular delivery of **vector**, there is hematogenous dissemination to the gonads and gradual washout of **vector** over time. Direct transduction of germ cells does not appear to occur (Arruda et al., Molecular Therapy, 2001). Based on these and other safety studies, the clinical trial has now resumed. A goal of this work will be to determine whether the therapeutic levels achieved in a large animal model of **hemophilia** can be realized in humans. Gene transfer holds promise as a therapeutic approach for a wide range of inherited and acquired diseases. Continued improvements in gene delivery systems will allow gene transfer strategies to move forward. It is worth recalling that, from the perspective of drug development, recombinant proteins once appeared extremely complex and difficult to standardize, yet today they are a standard part of the therapeutic armamentarium. Similarly, gene delivery vehicles, both viral and non-viral, represent a new level of complexity in therapeutics, yet recent data suggest that these too can be successfully developed for the treatment of human disease.

L7 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1995:893188 CAPLUS
DOCUMENT NUMBER: 124:26607
TITLE: **Gene therapy** for haemophilia
AUTHOR(S): Smith, Theodore A. G.
CORPORATE SOURCE: Genetic Therapy, Inc., Gaithersburg, MD, 20878, USA
SOURCE: Expert Opinion on Investigational Drugs (1995), 4(9), 833-42
CODEN: EOIDER; ISSN: 0967-8298
PUBLISHER: Ashley Publications
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review with 56 refs. Recently, there has been significant progress on the development of a **gene therapy** protocol for the treatment of **hemophilia**. **Expression** of physiol. levels of both human factors VIII and IX has been achieved in animal models, and correction of the disease phenotype has been demonstrated in **hemophilia B dogs**. Various durations of clotting factor **expression** in vivo have been obsd. Adenoviral **vector** -mediated **expression** of therapeutic levels of human **factor IX** has been sustained for at least ten months in mice. Although research achievements have been substantial, several **obstacles** impede progress toward clin. trials. Improvements in gene transfer vehicles and delivery methods are needed to ensure safe and efficacious **gene therapy**. Specific issues currently under investigation include the persistence of clotting factor **expression**, procedures which allow re-administration of therapy, and host immune responses to treatment.

	Hits	Search Text	DBs
1	0	sertoli with factor adj1 IX	USPAT
2	1570	factor adj1 IX	USPAT
3	35	(transform\$5 or transfect\$5) with sertoli	USPAT
4	3	((transform\$5 or transfect\$5) with sertoli) and (factor adj1 IX)	USPAT
5	6	selawry.in.	USPAT
6	17	selawry.in.	USPAT; US-PGPUB; EPO; JPO; DERWENT
7	58	immunologically adj1 privileged adj1 site	USPAT; US-PGPUB; EPO; JPO; DERWENT
8	43	(transform\$5 or transfect\$5) with sertoli adj1 cells	USPAT; US-PGPUB; EPO; JPO; DERWENT
9	4	13 and 14	USPAT; US-PGPUB; EPO; JPO; DERWENT
10	0	14 with factor adj1 (VIII or IX)	USPAT

	Time Stamp
1	2002/05/29 13:56
2	2002/05/29 13:57
3	2002/05/29 13:57
4	2002/05/29 13:57
5	2003/07/24 09:47
6	2003/07/24 09:47
7	2003/07/24 09:56
8	2003/07/24 09:57
9	2003/07/24 09:57
10	2003/07/24 10:02

L10 ANSWER 8 OF 20 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1994:200380 CAPLUS
DOCUMENT NUMBER: 120:200380
TITLE: Transplantation of microcapsules (a potential
bio-artificial organ): Biocompatibility and host
reaction
AUTHOR(S): Cole, D. R.; Waterfall, M.; McIntyre, M.; Baird, J. C.
CORPORATE SOURCE: Southampton Gen. Hosp., Southampton, S09 4XY, UK
SOURCE: Journal of Materials Science: Materials in Medicine
(1993), 4(5), 437-42
CODEN: JSMMEL; ISSN: 0957-4530
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Tolerance to alginate-polylysine-alginate microcapsules implanted into the peritoneal cavity was compared in the Wistar Furth rat and the BB/E (Wistar-derived) spontaneously diabetic rat. A marked foreign-body type reaction was obsd. in the BB/E rat in both diabetic and non-diabetic animals. In contrast, little or no reaction was obsd. in the Wistar Furth rat. Implantation under the kidney capsule, an **immunol. privileged site**, did not protect the microcapsules.

Blocking the surface charge of the microcapsule by coating with tolylene diisocyanate also failed to modify the reaction. Coating with a water-insol. lacquer (Eudragit RL) resulted in dense capsule overgrowth. Thus tolerance to alginate-polylysine-alginate microcapsules appears to be dependent upon the recipient animal strain and this may explain some of the discrepancies in function obsd. in different animal models when this system has been used to encapsulate pancreatic islets for a bioartificial **pancreas**. The tissue reaction does not seem to be affected by clin. diabetic status although abnormal immunol. responses in animals with a tendency to spontaneous diabetes could be important. Attempts to reduce the reaction to the capsules in the BB/E rat strain by modifying the membrane were unsuccessful.

=> d ibib abs 1-36

L7 ANSWER 1 OF 36 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 1
ACCESSION NUMBER: 2003:328838 CAPLUS
TITLE: Genetically modified NT2N human neuronal cells mediate long-term gene expression as CNS grafts *in vivo* and improve functional cognitive outcome following experimental traumatic brain injury
AUTHOR(S): Watson, Deborah J.; Longhi, Luca; Lee, Edward B.; Fulp, Carl T.; Fujimoto, Scott; Royo, Nicolas C.; Passini, Marco A.; Trojanowski, John Q.; Lee, Virginia M.-Y.; McIntosh, Tracy K.; Wolfe, John H.
CORPORATE SOURCE: Department of Pathobiology and Center for Comparative Medical Genetics, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA
SOURCE: Journal of Neuropathology and Experimental Neurology (2003), 62(4), 368-380
CODEN: JNENAD; ISSN: 0022-3069
PUBLISHER: American Association of Neuropathologists, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Human Ntera-2 (NT2) cells can be differentiated *in vitro* into well-characterized populations of NT2N neurons that engraft and mature when transplanted into the adult CNS of rodents and humans. They have shown promise as treatments for neurol. disease, trauma, and ischemic stroke. Although these features suggest that NT2N neurons would be an excellent platform for *ex vivo* gene therapy in the CNS, stable gene expression has been surprisingly difficult to achieve in these cells. In this report we demonstrate stable, efficient, and nontoxic gene transfer into undifferentiated NT2 cells using a pseudotyped lentiviral vector encoding the human elongation factor 1-.alpha. promoter and the reporter gene eGFP. Expression of eGFP was maintained when the NT2 cells were differentiated into NT2N neurons after treatment with retinoic acid. When transplanted into the striatum of adult nude mice, transduced NT2N neurons survived, engrafted, and continued to express the reporter gene for long-term time points *in vivo*. Furthermore, transplantation of NT2N neurons genetically modified to express nerve growth factor significantly attenuated cognitive dysfunction following traumatic brain injury in mice. These results demonstrate that defined populations of genetically modified human NT2N neurons are a practical and effective platform for stable ***ex vivo* gene delivery** into the CNS.
REFERENCE COUNT: 62 THERE ARE 62 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 2 OF 36 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:370493 CAPLUS
TITLE: Antibody targeted gene transfer to endothelium
AUTHOR(S): Tan, P. H.; Manunta, M.; Ardjomand, N.; Xue, S. A.; Larkin, D. F. P.; Haskard, D. O.; Taylor, K. M.; George, A. J. T.
CORPORATE SOURCE: Department of Immunology, Division of Medicine, Hammersmith Hospital, Imperial College London, London, W12 0NN, UK
SOURCE: Journal of Gene Medicine (2003), 5(4), 311-323
CODEN: JGMEFG; ISSN: 1099-498X
PUBLISHER: John Wiley & Sons Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Background One of the drawbacks of the currently available vectors for gene therapy is the lack of selectivity in gene delivery. We have therefore investigated a strategy to generate immunoliposomes to target non-viral vectors to cell surface receptors on endothelium. Materials and methods We have developed a novel method of coupling antibodies (Abs) to

liposomes complexed to DNA, using mild heat treatment to aggregate the IgG (IgG). The interaction of plasmid DNA, liposomes and Abs was measured using a gel retardation assay and a resonant mirror biosensor. The size of the transfection complex was detd. by light scattering, and the binding and internalization of the complex to cells was followed using flow cytometry. The transfection ability was tested on cell lines and primary cells in vitro and human corneal or vascular tissues ex vivo. Results The interaction of antibodies with liposomes is relatively stable ($t_{1/2}$.simeq. 45 min). The size of the liposome, Ab and DNA complex was found to be around 500 nm in 4% BSA. The addn. of anti-transferrin receptor Abs increased the internalization of the liposome-DNA complex into cells. Abs against both transferrin receptor and E-selectin were shown to augment transfection efficiency of liposomes to cell expressing the appropriate antigens. They are also shown to be efficient in mediating gene delivery to corneal and vascular tissues ex vivo. Conclusions We have shown that our novel vector is capable of in vitro and **ex vivo** gene delivery to cells and human tissues including cornea, artery and vein. In particular, an Ab against E-selectin was effective at selectively delivering genes to activated endothelial cells expressing the adhesion mol. Such a strategy will have applications for targeting these tissues prior to transplantation or autologous grafting, and, in the longer term, may allow in vivo targeting of gene therapy to inflammatory sites.

REFERENCE COUNT: 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 3 OF 36 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 2
ACCESSION NUMBER: 2003:392251 CAPLUS
TITLE: Efficient gene delivery to primary neuron cultures using a synthetic peptide vector system
AUTHOR(S): Collins, Louise; Asuni, Ayodeji A.; Anderton, Brian H.; Fabre, John W.
CORPORATE SOURCE: King's and St Thomas' School of Medicine, Guy's, Institute of Liver Studies, Department of Clinical Sciences, King's College Hospital, Bessemer Road, London, SE5 9PJ
SOURCE: Journal of Neuroscience Methods (2003), 125(1,2), 113-120
PUBLISHER: CODEN: JNMEDT; ISSN: 0165-0270
Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A bi-functional, 31 amino acid synthetic peptide (polylysine-molossin) was evaluated for gene delivery to primary cultures of rat cerebral cortex neurons. Polylysine-molossin consists of an amino terminal domain of 16 lysines for electrostatic binding of DNA, and a 15 amino acid, integrin-binding domain at the carboxyl terminal. High levels of gene delivery were obtained with 20-30 .mu.M chloroquine, with a synthetic fusogenic peptide at an optimal DNA:polylysine-molossin:fusogenic peptide wt./wt. ratio of 1:3:0.2, and with the addn. of low concns. of Lipofectamine 2000 at an optimal DNA:polylysine-molossin:Lipofectamine 2000 wt./wt. ratio of 1:3:0.5. With the best combination, >30% of neurons strongly expressed the .beta.-galactosidase reporter gene, with no observable toxicity. DNA concns. >2 .mu.g/mL were essential for efficient gene delivery. This synthetic peptide provides a safe, readily standardised and flexible DNA vector system well suited to **ex vivo** gene delivery to neurons for exptl. and clin. applications.

L7 ANSWER 4 OF 36 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 3
ACCESSION NUMBER: 2002:685022 CAPLUS
DOCUMENT NUMBER: 138:20149
TITLE: Evaluation of Tet-on system to avoid transgene

AUTHOR(S): down-regulation in ex vivo gene transfer to the CNS
Johansen, J.; Rosenblad, C.; Andsberg, K.; Moller, A.;
Lundberg, C.; Bjoerlund, A.; Johansen, T. E.
CORPORATE SOURCE: NsGene A/S, Ballerup, Den.
SOURCE: Gene Therapy (2002), 9(19), 1291-1301
CODEN: GETHEC; ISSN: 0969-7128
PUBLISHER: Nature Publishing Group
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Ex vivo gene transfer to the CNS has so far been hampered by instability of transgene expression. To avoid the phenomenon of transgene down-regulation, we have employed strong, constitutive promoters and compared this expression system with the inducible Tet expression system incorporated in a single plasmid vector or in lentiviral vectors. Plasmid-based transgene expression directed by the constitutive, human ubiquitin promoter, Ubc, was stable in transfected HiB5 cells in vitro and comparable in strength to the CMV promoter. However, after transplantation of Ubc and CMV HiB5 clones to the rat striatum, silencing of the transgene occurred in most cells soon after implantation of transfected cells. The Tet-on elements were incorporated in a single plasmid vector and inducible HiB5 clones were generated. Inducible clones displayed varying basal expression activity, which could not be ascribed to an effect of cis-elements in the vector, but rather was due, at least in part, to intrinsic activity of the minimal promoter. Basal expression activity could be blocked in a majority of cells by stable expression of the transrepressor tTS. Fully induced expression levels were comparable to CMV and Ubc promoters. Similar to the constitutive promoters transgene expression was down-regulated soon after grafting of inducible HiB5 clones to the rat striatum. Lentiviral vectors can direct long-term stable in vivo transgene expression. To take advantage of this quality of the lentiviral vector, the Tet-on elements were incorporated in two lentiviral transfer vectors followed by transduction of HiB5 cells. Interestingly, all HiB5 clones established by lentiviral transduction showed very similar expression patterns and tight regulatability that apparently was independent of transgene copy no. and integration site. Nevertheless, transgene expression in all lentiviral HiB5 clones was down-regulated shortly after transplantation to the rat striatum. These results confirm the general phenomenon of transgene down-regulation. Moreover, the results suggest that the considerable advantages offered by lentiviral vectors for direct gene delivery cannot necessarily be transferred directly to **ex vivo gene delivery**.

This emphasizes the need for alternative vector strategies for ex vivo gene transfer.

REFERENCE COUNT: 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 5 OF 36 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:417567 BIOSIS
DOCUMENT NUMBER: PREV200200417567
TITLE: Reversible model of spheroid formation allows for high efficiency of gene delivery ex vivo and accurate gene assessment in vivo.
AUTHOR(S): Alpaugh, Mary L.; Barsky, Sanford H. (1)
CORPORATE SOURCE: (1) Department of Pathology, UCLA School of Medicine, Los Angeles, CA, 90024: sbarsky@ucla.edu USA
SOURCE: Human Gene Therapy, (July 1, 2002) Vol. 13, No. 10, pp. 1245-1258. <http://www.liebertpub.com/HUM>. print.
ISSN: 1043-0342.

DOCUMENT TYPE: Article
LANGUAGE: English

AB The native three-dimensional architecture of carcinomas, which governs numerous autocrine-paracrine interactions related to tumor progression, cannot be faithfully recreated in most in vitro models. Even when the

three-dimensional architecture is recreated in artificial scaffolds such as soft agar, this approach does not truly recreate the natural microenvironment of the tumor. Multicellular spheroids can reasonably recreate *in vitro* the natural three-dimensional architecture of carcinomas, but even the most efficient gene delivery vectors will penetrate only the outer layers of these structures and hence only a small fraction of cells will receive the gene of interest. If the multicellular spheroids are disrupted into a single-cell suspension in order to achieve high transfection efficiency, the single-cell production may have so altered the gene expression profile of the spheroid as to bring into question its present relevancy to *in vivo* tumor progression. Our laboratory has developed a human-SCID (severe combined immunodeficient) mouse model of inflammatory breast cancer, MARY-X, which grows as tight multicellular spheroids *in vitro* and as lymphovascular emboli *in vivo*. The spheroids, which express only low levels of surface sialyl-Lewisx/a (sLex/a), are able to form compact homotypic cell clumps mediated by an intact, overexpressed E-cadherin/alpha,beta-catenin axis. The spheroids can be fully disrupted by trypsin proteolysis, anti-E-cadherin antibodies, or Ca²⁺ depletion. Of these approaches the disruption with depleted Ca²⁺, complete after 30 min, is fully reversible by the readdition of Ca²⁺ within 6 hr. This time interval allows for a transfection "window" in which successful gene delivery can be achieved before spheroid reformation. Retroviruses (106-107 CFU/ml) carrying the gene encoding either green fluorescent protein (GFP), a dominant-negative E-cadherin mutant (H-2Kd-E-cad), its control (H-2Kd-E-cadDELTAC25), or alpha-1,3-fucosyltransferase III (FucT-III), an enzyme that increases surface sLex/a, were used to transfect either intact (wild-type) or disadhered/readhered (reformed) spheroids. There were marked differences in transfection efficiency in the reformed versus wild-type spheroids. Retroviral transfection of GFP resulted in successful delivery of this reporter gene to only the outer layer of cells of the wild-type spheroids, but to all layers of the reformed spheroids. A single retroviral transfection of H-2Kd-E-cad, H-2Kd-E-cadDELTAC25, or FucT-III produced evidence of their respective gene expression at 72 hr throughout all layers of the reformed spheroids, but only H-2Kd-E-cad and FucT-III produced progressive disadherence. Both H-2Kd-E-cad-MARY-X and FucT-III-MARY-X lost their ability to form lymphovascular emboli in SCID mice. This reversible model of spheroid formation has provided us with insight into the pathogenesis of inflammatory breast carcinoma. If more broadly applied, this model could be used to examine the effects of any gene, using any gene delivery system in the three-dimensional context of native tumoral architecture.

L7 ANSWER 6 OF 36 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 4
ACCESSION NUMBER: 2002:845978 CAPLUS
DOCUMENT NUMBER: 137:368401
TITLE: **Ex vivo gene delivery of IL-1Ra and soluble TNF receptor confers a distal synergistic therapeutic effect in antigen-induced arthritis**
AUTHOR(S): Kim, Seon Hee; Lechman, Eric R.; Kim, Sunyoung; Nash, Joan; Oligino, Thomas J.; Robbins, Paul D.
CORPORATE SOURCE: Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA, 15261, USA
SOURCE: Molecular Therapy (2002), 6(5), 591-600
CODEN: MTOHCK; ISSN: 1525-0016
PUBLISHER: Elsevier Science
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Intra-articular expression of antagonists of interleukin-1.beta. (IL-1.beta.) and tumor necrosis factor-.alpha. (TNF-.alpha.) in arthritic rabbit knee and mouse ankle joints by direct adenoviral-mediated

intra-articular delivery results in amelioration of disease pathol. in both the treated and contralateral untreated joints. Previous expts. suggest that direct adenoviral infection of resident antigen-presenting cells (APCs) and subsequent traveling of these cells to other sites of inflammation and lymph nodes might be responsible for this "contralateral effect.". To det. whether genetic modification of APCs is required for the contralateral effect, the authors have used an ex vivo approach utilizing genetically modified fibroblasts to express IL-1 receptor antagonist protein (IL-1Ra) and sol. TNF-.alpha. receptor (sTNFR) locally in arthritic joints. Retroviral vectors carrying IL-1Ra, sTNFR-Ig, or both genes together were used to stably infect autologous rabbit fibroblasts that were then injected intra-articularly into arthritic rabbit knee joints. The intra-articular delivery of either IL-1Ra- or sTNFR-Ig-expressing fibroblasts was anti-inflammatory and chondro-protective in both the injected and noninjected contralateral joints. In addn., the authors demonstrate that the co-delivery of both antagonists in combination results in a synergistic effect in disease amelioration in both the treated and nontreated joints. These ex vivo results suggest that trafficking of vector-modified inflammatory cells is not the main mechanism responsible for the obsd. distal spread of the therapeutic effect. Moreover, the results demonstrate that local, ex vivo gene therapy for arthritis could be effective in blocking pathologies within untreated, distant arthritic joints.

REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 7 OF 36 CAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2002:935812 CAPLUS

DOCUMENT NUMBER: 138:378399

TITLE: Neuroprotective gene therapy for Parkinson's disease
Tenenbaum, L.; Chtarto, A.; Lehtonen, E.; Blum, D.; Baekelandt, V.; Velu, T.; Brotchi, J.; Levivier, M.

AUTHOR(S): Laboratory of Experimental Neurosurgery, Institut de Recherche Interdisciplinaire en Biologie Humaine et Moleculaire, Hopital Erasme, 808, Route de Lennik, Brussels, B-1070, Belg.

SOURCE: Current Gene Therapy (2002), 2(4), 451-483

CODEN: CGTUAH; ISSN: 1566-5232

PUBLISHER: Bentham Science Publishers Ltd.

DOCUMENT TYPE: Journal; General Review

LANGUAGE: English

AB A review. Parkinson's disease (PD) is a neurodegenerative disease characterized by a progressive loss of the dopaminergic neurons in the substantia nigra pars compacta. Accumulating evidence indicates that apoptosis contributes to neuronal cell death in PD patients' brain. Excitotoxicity, oxidative stress, and mitochondrial respiratory failure are thought to be the key inducers of the apoptotic cascade. Even though the initial cause and the mechanism of degeneration are poorly understood, neuroprotection can be achieved by interfering with neuronal cell death either directly or by preventing neuronal dysfunction. Potential agents for neuroprotection are neurotrophic factors, inhibitors of apoptosis or anti-oxidative agents. However, the existence of the blood-brain barrier precludes systemic delivery of these factors. In situ gene delivery provides strategies for local and sustained administration of protective factors at physiol. relevant doses. Viral vectors mediating stable gene expression in the central nervous system exist and are still under development. Efficacy of these vectors has repeatedly been demonstrated in the animal models both ex vivo and in vivo. **Ex vivo gene delivery** could furthermore be combined with cell replacement therapies by transplanting genetically modified cells compensating for the lost neuronal cell population to provide neuroprotection to both the grafted cells and degenerating host neurons. However, several aspects of gene transfer, such as uncontrolled diffusion,

axonal transport, unpredictable site of integration and immunological responses, still raise safety concerns and justify further development of viral and non-viral vectors as well as genetic elements with tightly controlled gene expression. Various relevant animal models for Parkinson's disease are available for the evaluation of gene therapy strategies. These include induction of cell death in specific neuron population through administration of toxins either directly in the brain or systemically, as well as transgenic mice expressing human disease-associated mutations.

REFERENCE COUNT: 355 THERE ARE 355 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE REFORMAT

L7 ANSWER 8 OF 36 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:733406 CAPLUS
DOCUMENT NUMBER: 138:296875
TITLE: Ex vivo gene therapy in the central nervous system
AUTHOR(S): Blesch, A.; Tuszynski, M. H.
CORPORATE SOURCE: Department of Neuroscience, University of California, La Jolla, CA, 92093-0626, USA
SOURCE: Handbook of Experimental Pharmacology (2002), 155(CNS Neuroprotection), 301-333
CODEN: HEPHD2; ISSN: 0171-2004
PUBLISHER: Springer-Verlag
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review. The targeted delivery of genes into the adult central nervous system (CNS) has received considerable interest in recent years with the development of improved viral vector systems and suitable strategies for therapeutic intervention. Exptl. gene therapy in animal models has been studied to prevent or slow the progression of chronic neurodegenerative diseases, to improve recovery after traumatic CNS injury and to kill malignant brain tumors. Genes that have been investigated in these various models include those that code for neurotrophic factors, neurotransmitter synthesis enzymes, modulators of neuronal and glial function, and inducers of cell death. Generally, two different types of gene therapy have been distinguished: *in vivo* and *ex vivo* gene therapy. The direct injection of genes into the CNS using viral vectors or DNA-liposome suspensions is termed *in vivo* gene therapy. *Ex vivo* gene therapy is based on genetic modification of cells *in vitro* followed by the grafting of these cells into the CNS. *Ex vivo* approaches to gene therapy will be the focus of this review. Gene therapy was classically viewed as a tool for the replacement of missing or defective genes. However, the delivery of pharmacol. amounts of therapeutic agents has also become a major focus of gene therapy since this approach provides a means of locally delivering therapeutic mols. to precise *in vivo* targets. This is esp. important in the CNS, where many diseases are restricted to specific, relatively small groups of cells. In these cases of localized cellular dysfunction, gene therapy has clear advantages over the systemic delivery of pharmaceuticals. Like every new form of therapeutic intervention, ***ex vivo* gene delivery** needs to be thoroughly evaluated for each specific application with regard to potential benefits and risk factors before starting clin. trials. To date, *ex vivo* gene therapy has been more extensively studied for therapeutic intervention and as a potential tool for elucidating CNS function than *in vivo* gene transfer. The base of knowledge regarding *ex vivo* approaches is large, and efficacy in a no. of CNS disease models has been established. However, direct *in vivo* gene delivery has some advantages over *ex vivo* gene transfer including a lower degree of invasiveness, a smaller risk of tumor formation, and a potentially reduced expense of treatment per patient.

REFERENCE COUNT: 163 THERE ARE 163 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE REFORMAT

FORMAT

L7 ANSWER 9 OF 36 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:546403 CAPLUS
DOCUMENT NUMBER: 138:67274
TITLE: Helper-dependent adenoviral vectors
AUTHOR(S): Zhou, Heshan; Pastore, Lucio; Beaudet, Arthur L.
CORPORATE SOURCE: Cell and Gene Therapy Center, Baylor Coll. Med.,
Houston, TX, 77030, USA
SOURCE: Methods in Enzymology (2002), 346 (Gene Therapy
Methods), 177-198
CODEN: MENZAU; ISSN: 0076-6879
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A strategy to reduce the immunogenicity and improve the safety of adenovirus (Ad) vectors used for *in vivo* and ***ex vivo*** gene delivery is to delete all viral coding sequences so that leaky expression of viral proteins is completely eliminated, although the proteins comprising the particle are still delivered. This type of vector depends on a helper virus to provide viral proteins in *trans*, leading to the terminol. of helper-dependent adenoviral (HD-Ad) vectors. HD-Ad vectors are substantially less toxic than other Ad vectors when high doses are administered intravascularly in mice. Initial results suggest that the HD-Ad vector system is one of the most promising viral vector system for gene therapy, particularly for achieving high level and long-term expression in the liver. (c) 2002 Academic Press.
REFERENCE COUNT: 49 THERE ARE 49 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 10 OF 36 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2003:315449 BIOSIS
DOCUMENT NUMBER: PREV200300315449
TITLE: TRANSPLANTATION OF BONE MARROW STROMAL CELLS (MSCS) AND BDNF - TRANSDUCED MSCS PROMOTES ROBUST AXONAL GROWTH AFTER SPINAL CORD INJURY.
AUTHOR(S): Lu, P. (1); Tuszynski, M. H. (1)
CORPORATE SOURCE: (1) Neurosciences, UCSD, La Jolla, CA, USA USA
SOURCE: Society for Neuroscience Abstract Viewer and Itinerary Planner, (2002) Vol. 2002, pp. Abstract No. 634.11.
<http://sfn.scholarone.com>. cd-rom.
Meeting Info.: 32nd Annual Meeting of the Society for Neuroscience Orlando, Florida, USA November 02-07, 2002
Society for Neuroscience

DOCUMENT TYPE: Conference
LANGUAGE: English
AB Bone marrow stromal cells (MSCs) are the stem cells and progenitors of skeletal tissue. Recent studies indicate that MSCs can be chemically induced to become neuron-like cells *in vitro*. Therefore, MSCs offer the potential to replace lost tissue after nervous system injury. This study investigated whether MSCs could differentiate into cells with neuronal characteristics *in vivo* when grafted into sites of spinal cord injury, and establish matrices supportive of host axonal growth into the lesion site. Further, we examined whether MSCs, genetically modified to over-express BDNF, could enhance host axonal growth. *In vitro* prior to grafting, rat MSCs were chemically induced to become neuron-like cells expressing the neuronal markers NSE and, occasionally, MAP-1B. One month after grafting to cystic dorsal column lesions in the cervical spinal cord of 8 adult rats, neuron-like MSCs supported growth of host sensory and motor axons; however, on phenotypic analysis, the MSCs no longer exhibited neuronal features *in vivo*. Grafts of BDNF-transduced MSCs (n = 8 animals) exhibited a significant increase in the extent and diversity of host axonal growth,

also attracting the growth of host serotonergic, coeruleospinal and dorsal column sensory axons. Additional analysis is underway to examine functional outcomes. Thus, MSCs can support host axonal growth after spinal cord injury and are suitable cell types for **ex vivo** gene delivery.

L7 ANSWER 11 OF 36 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2003:282422 BIOSIS
DOCUMENT NUMBER: PREV200300282422
TITLE: LONG - TERM **EX VIVO GENE**
DELIVERY OF GDNF USING PRIMARY ASTROCYTES
TRANSDUCED WITH A LENTIVIRAL VECTOR.
AUTHOR(S): Ericson, C. (1); Georgievska, B. (1); Kirik, D. (1);
Lundberg, C. (1)
CORPORATE SOURCE: (1) Dept Physiological Sci, Wallenberg Neurosci Ctr, Lund,
Sweden Sweden
SOURCE: Society for Neuroscience Abstract Viewer and Itinerary
Planner, (2002) Vol. 2002, pp. Abstract No. 165.17.
<http://sfn.scholarone.com>. cd-rom.
Meeting Info.: 32nd Annual Meeting of the Society for
Neuroscience Orlando, Florida, USA November 02-07, 2002
Society for Neuroscience

DOCUMENT TYPE: Conference
LANGUAGE: English

AB Primary astrocytes derived from E15 rat lateral ganglionic eminence (LGE) were genetically modified to express glial cell line-derived neurotrophic factor (GDNF) by using a recombinant lentiviral vector. In previous studies, the astrocytes have shown a great potential for use in *ex vivo* gene therapy to the rat striatum (Ericson et al 2002). GDNF is a very potent neurotrophic factor and has survival promoting effects on dopaminergic (DA) neurons. Before transplantation, the astrocytes released 27 ng GDNF/100.000 cells/hour in culture. The GDNF-expressing astrocytes were transplanted into the striatum of intact adult rats (300.000 cells) and the tissue levels of GDNF were analyzed by ELISA at 1 week and 4 weeks after transplantation. The level of GDNF expression was 2.93+-0.62 ng/mg tissue at 1 week and 0.42+-0.27 ng/mg tissue at 4 weeks. No detectable expression of GDNF was observed in animals transplanted with native astrocytes. The expression of GDNF in the striatum caused an enhanced amphetamine-induced contralateral turning at 1 week (about 5 turns/min), indicating an upregulation of the DA system in response to GDNF. This turning bias was decreased at 4 weeks (about 2 turns/min) and lost at 8 weeks after grafting. The GDNF-expressing astrocytes will be transplanted to the striatum or substantia nigra prior to an intrastriatal 6-hydroxydopamine lesion in order to study the neuroprotective effects in this rat model of Parkinson's disease.

L7 ANSWER 12 OF 36 CAPLUS COPYRIGHT 2003 ACS on STNDUPPLICATE 5
ACCESSION NUMBER: 2001:934020 CAPLUS
DOCUMENT NUMBER: 136:49320
TITLE: Method for targeted delivery of nucleic acids
INVENTOR(S): Filpula, David R.; Wang, Maoliang; Whitlow, Marc D.
PATENT ASSIGNEE(S): Enzon, Inc., USA
SOURCE: U.S., 39 pp.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6333396	B1	20011225	US 1999-420592	19991019

US 2002151061	A1	20021017	US 2001-983580	20011025
US 2002156248	A1	20021024	US 2001-985442	20011102
PRIORITY APPLN. INFO.:			US 1998-104949P	P 19981020
			US 1999-420592	A3 19991019

AB The present invention is directed to a method of in vivo and **ex vivo gene delivery**, for a variety of cells. More specifically, it relates to a novel carrier system and method for targeted delivery of nucleic acids to mammalian cells. More specifically, the present invention relates to carrier system comprising single-chain polypeptide binding mols. having an a region rich in basic amino acid and having the three dimensional folding and, thus, the binding ability and specificity, of the variable region of an antibody. The basic amino acid rich region can comprise oligo-lysine, oligo-arginine or combinations thereof. Such preps. of modified single chain polypeptide binding mols. also have ability to bind nucleic acids at the region rich in basic amino acid residues. These properties of the modified single chain polypeptide binding mols. make them very useful in a variety of therapeutic applications including gene therapy. The invention also relates to multivalent antigen-binding mols. having regions rich in basic amino acids. Compns. of, genetic constructions for, methods of use, and methods for producing basic amino acid tailed antigen-binding proteins are disclosed.

REFERENCE COUNT: 115 THERE ARE 115 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 13 OF 36 CAPLUS COPYRIGHT 2003 ACS on STN
 ACCESSION NUMBER: 2001:661568 CAPLUS
 DOCUMENT NUMBER: 135:238607
 TITLE: Cloning, characterization and therapeutic applications of ARTS-1, sheddase of TNF type I receptor and other cytokine receptors
 INVENTOR(S): Levine, Stewart
 PATENT ASSIGNEE(S): Government of the United States of America, as Represented by the Secretary, Department of Health and Human Services, USA
 SOURCE: PCT Int. Appl., 139 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001064856	A2	20010907	WO 2001-US6464	20010228
WO 2001064856	A3	20020418		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 2001045371	A5	20010912	AU 2001-45371	20010228
PRIORITY APPLN. INFO.:			US 2000-185586P	P 20000228
			WO 2001-US6464	W 20010228

AB The present invention provides compns. and methods for the regulation of cytokine signaling through the tumor necrosis factor (TNF) pathway. Specifically, the invention provides a novel gene, polypeptide and related compns. and methods for the regulation of ectodomain shedding.

Specifically, the invention provides a novel polypeptide and a gene which encodes the polypeptide, which has the ability to promote the shedding of the extracellular domain of type I TNF receptor (TNFR1). This polypeptide and gene are called ARTS-1, for aminopeptidase regulator of type I, 55 kDa TNF receptor ectodomain shedding. Cloning, amino acid and encoding cDNA sequences of human ARTS-1 are disclosed. The open reading frame predicted from the human ARTS-1 cDNA encodes a protein of 941 amino acid residues. The patterns of tissue expression of the endogenous ARTS-1 and recombinant ARTS-1 expression in cultured cell lines are described. ARTS-1 TNFR1 ectodomain sheddase regulatory activity is analyzed. It is contemplated that ARTS-1 will also regulate the shedding of ectodomains of other cytokine receptors including IL-1RII and IL-6R. In preferred embodiments, methods and compns. for the regulation of TNFR1 ectodomain shedding are provided. The present invention finds use in therapeutics, diagnostics, and drug screening applications.

L7 ANSWER 14 OF 36 CAPLUS COPYRIGHT 2003 ACS on STN
 ACCESSION NUMBER: 2001:265436 CAPLUS
 DOCUMENT NUMBER: 134:291102
 TITLE: Temperature-sensitive regulation of viral vector integration and production with Rep protein mutant for gene delivery and therapy
 INVENTOR(S): Samulski, Richard Jude; Gavin, Denise; Muzycka, Nicholas; Abernathy, Corinne; Pereira, Daniel
 PATENT ASSIGNEE(S): The University of North Carolina At Chapel Hill, USA; University of Florida
 SOURCE: PCT Int. Appl., 92 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001025253	A2	20010412	WO 2000-US26916	20000929
WO 2001025253	A3	20010614		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 2000077390	A5	20010510	AU 2000-77390	20000929
PRIORITY APPLN. INFO.:			US 1999-157245P	P 19991001
			US 1999-157248P	P 19991001
			WO 2000-US26916	W 20000929

AB The present invention provides temp.-sensitive (ts) adeno-assocd. virus (AAV) Rep78 and Rep68 proteins. In preferred embodiments, the ts AAV Rep78 and Rep68 proteins have missense mutations at amino acid positions 40, 42 and 44 (Asp.fwdarw.Ala) that confer a temp.-sensitive phenotype. Also provided are nucleotide sequences and vectors encoding the inventive ts Rep proteins. A hybrid adenovirus vector is provided that stably comprises a nucleotide sequence encoding a ts AAV Rep protein according to the invention. The present invention also provides methods of packaging AAV vectors and methods of **ex vivo gene delivery** using the ts Rep proteins of the invention. Further provided are cells contg. the ts AAV Rep proteins, preferably stably integrated into the genome of the cell at permissive temps. The cells may then be shifted to non-permissive temps. to inactivate the ts Rep

protein(s), thereby mitigating concerns regarding the presence of functional Rep protein in target cells as well as potential toxicity to target cells.

L7 ANSWER 15 OF 36 CAPLUS COPYRIGHT 2003 ACS on STNDUPPLICATE 6
ACCESSION NUMBER: 2003:115027 CAPLUS
TITLE: Gene therapy in rheumatic diseases
AUTHOR(S): Vervoordeldonk, Margriet J. B. M.; Tak, Paul P.
CORPORATE SOURCE: Division of Clinical Immunology and Rheumatology,
Department of Medicine, Academic Medical Centre,
University of Amsterdam, Amsterdam, 1100 DD, Neth.
SOURCE: Best Practice & Research, Clinical Rheumatology
(2001), 15(5), 771-788
CODEN: BPRCC7
PUBLISHER: Bailliere Tindall
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by joint inflammation as well as progressive cartilage and bone destruction. Advances in the understanding of the pathophysiol. of RA have led to the development of new therapeutic strategies, including gene therapy. Gene therapy offers a new approach to deliver therapeutic proteins to the joints of arthritis patients. Local as well as systemic gene therapy can be envisaged for the treatment of arthritis. Several viral and non-viral vectors have been used in animal models for rheumatoid arthritis for ex vivo and in vivo delivery of therapeutic genes. Promising pre-clin. data have resulted from the application of these strategies. Using **ex vivo gene delivery**, successful and safe gene transfer has been demonstrated in the joints of RA patients. Although new insights into the role of cytokines and other mediators of chronic inflammation have provided novel targets for therapeutic intervention, the development of vectors that induce long-term and regulated gene expression remains a challenge.
REFERENCE COUNT: 67 THERE ARE 67 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 16 OF 36 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 7
ACCESSION NUMBER: 2002:167119 BIOSIS
DOCUMENT NUMBER: PREV200200167119
TITLE: Nonviral transfection of intact pancreatic islets.
AUTHOR(S): Lakey, J. R. T. (1); Young, A. T. L.; Pardue, D.; Calvin,
S.; Albertson, T. E.; Jacobson, L.; Cavanagh, T. J.
CORPORATE SOURCE: (1) Surgical-Medical Research Institute, University of
Alberta, 1074 Dentistry/Pharmacy Building, Edmonton,
Alberta, T6G 2N8: jonathan.lakey@ualberta.ca Canada
SOURCE: Cell Transplantation, (2001) Vol. 10, No. 8, pp. 697-708.
print.
ISSN: 0963-6897.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Ex vivo gene transfer offers a potential means to introduce genes into cells, which may play an important role in preventing graft rejection and inducing graft tolerance. This study examined the efficiency and toxicity of several lipid-based transfection reagents (LipofectAMINE, DOTAP, and DOSPER) in intact pancreatic islets. Isolated islets were transfected with a pCMV-beta-galactosidase plasmid using several DNA/liposome ratios (1:12) of liposomes (3-72 μ l) and DNA (3 and 6 μ g). Transfection efficiency was quantified by microscopic evaluation of beta-galactosidase gene expression in whole intact islets. Functionality of the transfected islets was measured by insulin response to glucose solutions. All transfection reagents evaluated in this study transfected cells within the islets. As expected, untransfected controls and transfected islets with DNA alone did

not express beta-gal. The highest transfection efficiency and functional viability were obtained following a 48-h incubation after exposure to the transfection mixtures as follows: 3 μ g DNA and 18 μ g DOTAP/ml (1:6 ratio), 6 μ g DNA and 12 μ g DOSPER/ml (1:2 ratio), or 6 μ g DNA and 12 μ g LipofectAMINE/ml (1:2 ratio). The highest rate of transfected cells per islet was obtained using DOTAP. In vitro functionality was not significantly different between DOTAP and nontreated controls. However, optimal transfection efficiency doses of LipofectAMINE and DOSPER significantly reduced the stimulated insulin response of the transfected islets ($p < 0.05$, ANOVA). The calculated stimulation index (SI) was 7.8 ± 0.6 (mean \pm SEM) for DOTAP-transfected islets compared with 8.4 ± 0.5 for nontransfected control islets ($p = ns$). The SI of DOSPER- and LipofectAMINE-transfected islets was significantly lower (6.1 ± 0.5 and 3.4 ± 0.5 , respectively, $p < 0.05$). Lipid-based transfection using DOTAP at a DNA/lipid ratio of 1:6 provides an effective means of **ex vivo gene delivery** without compromising in vitro functionality of the transfected islets.

L7 ANSWER 17 OF 36 CAPLUS COPYRIGHT 2003 ACS on STNDUPPLICATE 8
ACCESSION NUMBER: 2001:570976 CAPLUS
DOCUMENT NUMBER: 135:298984
TITLE: GDNF gene delivery to injured adult CNS motor neurons promotes axonal growth, expression of the trophic neuropeptide CGRP, and cellular protection
AUTHOR(S): Blesch, Armin; Tuszyński, Mark H.
CORPORATE SOURCE: Department of Neurosciences-0626, University of California, La Jolla, CA, 92093, USA
SOURCE: Journal of Comparative Neurology (2001), 436(4), 399-410
CODEN: JCNEAM; ISSN: 0021-9967
PUBLISHER: Wiley-Liss, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Glial-cell-line-derived neurotrophic factor (GDNF) has been identified as a potent survival and differentiation factor for several neuronal populations in the central nervous system (CNS), but to date, distinct effects of GDNF on motor axon growth and regeneration in the adult have not been demonstrated. In the present study, **ex vivo gene delivery** was used to directly examine whether GDNF can influence axonal growth, expression of neuronal regeneration-related genes, and sustain the motor neuronal phenotype after adult CNS injury. Adult Fischer 344 rats underwent unilateral transections of the hypoglossal nerve, followed by intramedullary grafts of fibroblasts genetically modified to secrete GDNF. Control animals received lesions and grafts of cells expressing a reporter gene. Two weeks later, GDNF gene delivery robustly promoted the growth of lesioned hypoglossal motor axons, altered the expression and intracellular trafficking of the growth-related protein calcitonin gene-related peptide (CGRP), and significantly sustained the cholinergic phenotype in $84 \pm 6\%$ of hypoglossal neurons compared with $39 \pm 6\%$ in control animals ($P < 0.001$). This is the first neurotrophic factor identified to increase the *in vivo* expression of the trophic peptide CGRP and the first report that GDNF promotes motor axonal growth *in vivo* in the adult CNS. Taken together with previous *in vitro* studies, these findings serve as the foundation for a model wherein GDNF and CGRP interact in a paracrine manner to regulate neuromuscular development and regeneration.
REFERENCE COUNT: 58 THERE ARE 58 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 18 OF 36 CAPLUS COPYRIGHT 2003 ACS on STNDUPPLICATE 9
ACCESSION NUMBER: 2000:515156 CAPLUS
DOCUMENT NUMBER: 133:359601
TITLE: A herpesvirus saimiri-based gene therapy vector with

AUTHOR(S) : potential for use in cancer immunotherapy
Stevenson, Alex J.; Frolova-Jones, Elena; Hall,
Kersten T.; Kinsey, Sally E.; Markham, Alexander F.,
Whitehouse, Adrian; Meredith, David M.

CORPORATE SOURCE: Molecular Medicine Unit, St. James's University Hospital, University of Leeds, Leeds, LS9 7TF, UK

SOURCE: Cancer Gene Therapy (2000), 7(7), 1077-1085

CODEN: CGTHEG; ISSN: 0929-1903

PUBLISHER: Nature America Inc.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The herpesvirus saimiri (HVS) genome has the capacity to incorporate large amounts of heterologous DNA and can be maintained episomally in many different human cell types. To evaluate the efficacy of HVS-mediated gene transfer into human hemopoietic cells, we investigated the ability of an HVS-based construct, carrying the enhanced green fluorescent protein (EGFP) and neomycin resistance genes, to transduce a variety of human hemopoietic cell lines and primary CD34+ cells. As measured by flow cytometry, the nos. of EGFP+ cells at 2 days postinfection differed between various cell types ranging, from 1.3% for KG1 cells to 56.8% for THP-1 cells. In addn., the expression of EGFP in Jurkat cells was retained at >95% per round of cell division over a period of 6 wk (comparable with Epstein-Barr virus-derived gene therapy systems). Although the virus was not specifically disabled, no lytic viral mRNAs could be detected in transduced Jurkat cells, and infectious virus could not be detected by sensitive virus recovery assay. We also describe a simple centrifugation method that increases the efficiency of transduction by > 100% in some cases and may be generally applicable to other herpesvirus-based vectors for **ex vivo gene delivery**. Using this technique, we were able to demonstrate a tropism for CD34+/CD14+ cells, transducing 30% of the population. These cells are known to give rise to dendritic cells (the most potent of the antigen-presenting cells), suggesting that the vector could be used to deliver DNA sequences encoding tumor antigens for cancer immunotherapy.

REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 19 OF 36 CAPLUS COPYRIGHT 2003 ACS on STNDUPPLICATE 10

ACCESSION NUMBER: 2000:878341 CAPLUS

DOCUMENT NUMBER: 134:172862

TITLE: Gene therapy for attenuating cardiac allograft arteriopathy using ex vivo E2F decoy transfection by HVJ-AVE-liposome method in mice and nonhuman primates

AUTHOR(S) : Kawauchi, Motohiro; Suzuki, Jun-Ichi; Morishita, Ryuichi; Wada, Yuko; Izawa, Atsushi; Tomita, Naruya; Amano, Jun; Kaneda, Yasufumi; Ogihara, Toshio; Takamoto, Shinichi; Isobe, Mitsuaki

CORPORATE SOURCE: Department of Cardiovascular Surgery, Faculty of Medicine, University of Tokyo, Tokyo, Japan

SOURCE: Circulation Research (2000), 87(11), 1063-1068

CODEN: CIRUAL; ISSN: 0009-7330

PUBLISHER: Lippincott Williams & Wilkins

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Cardiac allograft arteriopathy, which limits the long-term survival of recipients, is characterized by diffuse intimal thickening composed of proliferative smooth muscle cells. The transcription factor E2F plays a pivotal role in the coordinated transcription of cell-cycle regulatory genes. To test the hypothesis that double-stranded DNA with specific affinity for E2F (E2F decoy) is effective in preventing intimal hyperplasia, the authors performed ex vivo single intraluminal delivery of E2F decoy into cardiac allografts of mice and Japanese monkeys using the hemagglutinating virus of Japan (HVJ) artificial viral envelope-liposome

method. In murine models, antisense cyclin-dependent kinase 2 (cdk2) kinase oligodeoxynucleotide (ODN) and no transfers were performed to compare the effects. Severe intimal thickening was obsd., and multiple cell-cycle regulatory genes were enhanced in untreated allografts. E2F decoy prevented neointimal formation and suppressed these genes for 1 to < 8 wk, whereas antisense cdk2 kinase ODN had limited effects. In primate models, E2F decoy dramatically prevented neointimal thickening and suppressed multiple cell-cycle regulatory genes, whereas intimal thickening developed in the nontransfected or mismatch decoy-transfected allografts. Gel mobility shift assay proved the specific effects of E2F decoy, and reverse transcriptase-polymerase chain reaction documented that neither complication nor dissemination of HVJ into other organs was obsd. The authors demonstrate that **ex vivo gene delivery** to allografts is a potent strategy to modify allograft gene expression, resulting in prevention of graft arteriopathy without systemic adverse effects.

REFERENCE COUNT: 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 20 OF 36 CAPLUS COPYRIGHT 2003 ACS on STNDUPPLICATE 11
ACCESSION NUMBER: 2000:297839 CAPLUS
DOCUMENT NUMBER: 133:12721
TITLE: **Ex vivo gene delivery** using an adenovirus vector in treatment for cartilage defects
AUTHOR(S): Ikeda, Takumi; Kubo, Toshikazu; Nakanishi, Tohru; Arai, Yuji; Kobayashi, Kappei; Mazda, Osam; Ohashi, Suzuyo; Takahashi, Kenji; Imanishi, Jiro; Takigawa, Masaharu; Hirasawa, Yasusuke
CORPORATE SOURCE: Department of Orthopaedic Surgery, Kyoto Prefectural University of Medicine, Kyoto, 602-8566, Japan
SOURCE: Journal of Rheumatology (2000), 27(4), 990-996
CODEN: JRHUA9; ISSN: 0315-162X
PUBLISHER: Journal of Rheumatology Publishing Co. Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB To realize local selective gene expression in grafted chondrocytes for cartilage defect, the authors investigated the usefulness of an **ex vivo gene delivery** method using an adenovirus vector. β -Galactosidase gene (LacZ) was transfected using an adenovirus vector to chondrocytes isolated from rat joints. The cells were then embedded into collagen gel, and LacZ expression in the gel was examd. using 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-gal) staining; β -galactosidase activity was also measured. The collagen gel contg. transfected chondrocytes was grafted to the exptl. cartilage defects, and the expression of delivered gene was histol. examd. after X-gal staining of the tissue contg. the grafted area. X-gal pos. chondrocytes in the gel accounted for 82% at 1 wk and 55% at 8 wk after gene delivery. β -galactosidase activity decreased with time, but its expression was maintained even at 8 wk after gene delivery. Chondrocytes used in the allograft maintained their morphol., and the expression of delivered gene continued during the 8 wk period. In this **ex vivo** method, delivered gene can be expressed efficiently for a long time; this method would be useful in allografts for cartilage defects.

REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 21 OF 36 CAPLUS COPYRIGHT 2003 ACS on STM
ACCESSION NUMBER: 2001:77367 CAPLUS
DOCUMENT NUMBER: 134:256727
TITLE: Gene delivery into rat glomerulus using a mesangial cell vector
AUTHOR(S): Kim, Hey Jin; Kim, Sung Il; Yun, Ik Jin; Kwak, Joon

CORPORATE SOURCE: Hyeok; Yu, Suk Hee
Hyonam Kidney Laboratory, Soon Chun Hyang University,
Seoul, 140-743, S. Korea
SOURCE: Molecules and Cells (2000), 10(6), 662-668
CODEN: MOCEEK; ISSN: 1016-8478
PUBLISHER: Springer-Verlag Singapore Pte. Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB To develop an effective protocol of gene transfer into glomeruli, an **ex vivo gene delivery** system using rat mesangial cells (RMC) as a vector was examined. RMC genetically engineered with a retrovirus harboring the *Escherichia coli* β -galactosidase gene was used to test the efficacy of gene delivery and the location of the cells within the kidney. The RMC expressing β -galactosidase, RMCLZ1, was cultured *in vitro* and the cells were injected into the left kidney through the renal artery of a normal Sprague Dawley rat. At least 1. times. 106 RMCLZ1 was required for effective gene delivery into glomeruli. One hour and 1, 4, and 14 d after injection, glomeruli were isolated from the left kidneys injected with the cells and the expression of β -galactosidase in each glomeruli was evaluated. One hour and 1 d after injection, more than 90 and 80%, resp., of glomeruli from the left kidney showed strong β -galactosidase activity, while no activity of β -galactosidase was found in the glomeruli from the right kidneys. The no. of glomeruli stained by X-gal and the intensity decreased with time. Fourteen days after injection, about 35% of the glomeruli retained the RMCLZ1. X-gal and periodic acid-Schiff staining of frozen sections obtained 14 d after injection allowed the test. of the site where the mesangial cells injected were located. The mesangial cells were found mainly in two different locations, the glomerular capillary and the mesangium. The majority (about 90%) of the mesangial cells were located in the glomerular capillary and about 9% of the cells were in the mesangial area. Occasionally, the pos. staining was found in proximal tubules and the interlobular artery. Although addnl. methods are required for the site-specific targeting of the mesangial area, the **ex vivo** gene transfer to glomeruli is feasible and may be a useful tool for future investigations in the pathol. mechanisms of glomerular injury.

REFERENCE COUNT: 15 THERE ARE 15 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 22 OF 36 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:339201 CAPLUS
DOCUMENT NUMBER: 135:247066
TITLE: In vitro and **ex vivo gene delivery** to cells by electroporation
AUTHOR(S): Hui, Sek Wen; Li, Lin Hong
CORPORATE SOURCE: Molecular and Cellular Biophysics Department, Roswell Park Cancer Institute, Buffalo, NY, USA
SOURCE: Methods in Molecular Medicine (2000), 37, 157-171
CODEN: MMMEFN
PUBLISHER: Humana Press Inc.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review with 33 refs. describes electroporation as a means to transfer genetic materials to cells *in vitro* and *ex vivo*, including descriptions of the various factors governing electroporation and recovery after electroporation.

REFERENCE COUNT: 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 23 OF 36 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:161239 CAPLUS
DOCUMENT NUMBER: 135:170528

TITLE: Gene delivery with polyethylenimine
AUTHOR(S): Zou, S. M.; Behr, Jean-Paul; Goula, D.; Demeneix, Barbara
CORPORATE SOURCE: Laboratoire de Chimie Genetique, Illkirch, Fr.
SOURCE: Gene Therapy (2000), 131-139. Editor(s): Templeton, Nancy Smyth; Lasic, Danilo D. Marcel Dekker, Inc.: New York, N. Y.
CODEN: 69BAIG
DOCUMENT TYPE: Conference; General Review
LANGUAGE: English
AB A review with 27 refs. Topics discussed include **ex vivo gene delivery**; **in vivo delivery**; delivery to the central nervous system; i.v. delivery; and future directions. Polyethylenimine is clearly a most promising vector for gene delivery in a no. of **ex vivo** and **in vivo** settings. It is shown that correct formulation allows for control of DNA condensation and size of the resulting complexes. These factors will in turn affect not only interaction with the cell membrane and release from the endosome, but also intracellular trafficking and possibly nuclear entry, dissociation of complexes, and gene expression.
REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 24 OF 36 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:161236 CAPLUS
DOCUMENT NUMBER: 135:161944
TITLE: Ex vivo gene therapy using myoblasts and regulatable retroviral vectors
AUTHOR(S): Ozawa, Clare R.; Springer, Matthew L.; Blau, Helen M.
CORPORATE SOURCE: Stanford University School of Medicine, Stanford, CA, USA
SOURCE: Gene Therapy (2000), 61-80. Editor(s): Templeton, Nancy Smyth; Lasic, Danilo D. Marcel Dekker, Inc.: New York, N. Y.
CODEN: 69BAIG
DOCUMENT TYPE: Conference; General Review
LANGUAGE: English
AB A review with 145 refs. Topics discussed include the skeletal muscle as a target tissue for gene therapy; the development of **ex vivo gene delivery** by myoblast transplantation; the applications of myoblast-mediated **ex vivo gene delivery**; disease targets for myoblast-mediated gene transfer; regulatable retroviral vectors and the RetroTet-ARTsystem; and future prospects for myoblast-mediated therapies.
REFERENCE COUNT: 145 THERE ARE 145 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 25 OF 36 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2000:659299 CAPLUS
DOCUMENT NUMBER: 133:358784
TITLE: Gene therapy of neurodegenerative and demyelinating diseases
AUTHOR(S): Tenenbaum, Liliane
CORPORATE SOURCE: Laboratory of Experimental Neurosurgery Institut de Recherche Interdisciplinaire en Biologie Humaine et Nucleaire Universite Libre de Bruxelles, Brussels, B-1070, Belg.
SOURCE: NATO Science Series, Series A: Life Sciences (2000), 323(Targeting of Drugs: Strategies for Gene Constructs and Delivery), 53-68
CODEN: NASAF2; ISSN: 1387-6686
PUBLISHER: IOS Press
DOCUMENT TYPE: Journal; General Review

LANGUAGE: English
AB A review with 74 refs. Neurodegenerative diseases (NDD) such as Parkinson's disease (PD), Alzheimer disease, amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD) are characterized by neuronal cell death. Demyelinating diseases (DMD) such as multiple sclerosis (MS) or Charcot-Marie-Tooth (CMT) disease are characterized by degeneration of oligodendrocytes (the myelin-forming cells of the central nervous system) or Schwann cells (the myelin-forming cells of the peripheral nervous system). Current therapies only provide symptomatic amelioration and are not curative. Neuroscience research has led to the identification of genes involved in neuronal or myelin-forming cell differentiation, death and survival and in some cases in genetic forms of these diseases. Even when the initial cause of degeneration is unknown, nervous system recovery can be promoted by cell replacement or gene delivery. Candidate genes are those protecting or rescuing degenerating cells (involving antiapoptotic genes, genes coding for trophic factors, antioxidant enzymes, etc..) or coding for compensatory enzymes. Various animal models mimicking neurodegenerative or demyelinating diseases have been developed and consist in e.g. inducing specific cell death either by injection of toxins directly in the central nervous system or by systemic administration. When available, mutant or transgenic mice can also be used. Gene delivery includes direct *in vivo* delivery using recombinant viruses or DNA complexed with non viral vectors such as liposomes, polyethylene-imine, etc.. and **ex vivo gene delivery** using heterologous or allogenous genetically-modified cells transplanted in the damaged area. Examples covering various animal models and therapeutic strategies will be given, with the aim to illustrate specific advantages and disadvantages of viral and non-viral vectors in clin. relevant situations.

REFERENCE COUNT: 74 THERE ARE 74 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 26 OF 36 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 2000096842 EMBASE
TITLE: Insights into the molecular pathogenesis of atherosclerosis and therapeutic strategies using gene transfer.
AUTHOR: Hiltunen M.O.; Turunen M.P.; Laitinen M.; Yla-Herttula S.
CORPORATE SOURCE: S. Yla-Herttula, Department of Molecular Medicine, AI Virtanen Institute, University of Kuopio, PO Box 1627, FIN-70211 Kuopio, Finland
SOURCE: Vascular Medicine, (2000) 5/1 (41-48).
Refs: 129
ISSN: 1358-863X CODEN: VAMLFP
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 005 General Pathology and Pathological Anatomy
018 Cardiovascular Diseases and Cardiovascular Surgery
022 Human Genetics

LANGUAGE: English
SUMMARY LANGUAGE: English
AB Gene therapy for the treatment of atherosclerosis and related diseases has shown its potential in animal models and in the first human trials. Gene transfer to the vascular system can be performed both via intravascular and extravascular periadventitial routes. Intravascular gene transfer can be done with several types of catheters under fluoroscopic control. Extravascular gene transfer, on the other hand, provides a well-targeted gene delivery route available during vascular surgery. It can be done with direct injection or by using perivascular cuffs or surgical collagen sheets. **Ex vivo gene delivery** via transfected smooth muscle cells or endothelial cells might be useful for the production of secreted therapeutic compounds. Gene transfer to the liver has been used for the treatment of hyperlipidemia. The first clinical trials for the induction of therapeutic angiogenesis in ischemic

myocardium or peripheral muscles with VEGF or FGF gene transfer are under way and preliminary results are promising. VEGF has also been used for the prevention of postangioplasty restenosis because of its capability to induce endothelial repair and production of NO and prostacyclin. However, further basic research is needed to fully understand the pathophysiological mechanisms involved in conditions related to atherosclerosis. Also, further development of gene transfer vectors and gene delivery techniques will improve the efficacy and safety of human gene therapy.

L7 ANSWER 27 OF 36 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1999:57362 BIOSIS
DOCUMENT NUMBER: PREV199900057362
TITLE: HSV-1 vector for direct and **ex vivo** gene delivery.
AUTHOR(S): Bloom, D. C. (1); Tran, R. K.; Peng, Hu; Stachowiak, E. K.; Vu, E. T.; Stachowiak, M. K.
CORPORATE SOURCE: (1) Mol. and Structural Neurobiol. and Gene Ther. Program, Ariz. State Univ., Tempe, AZ 85287 USA
SOURCE: Society for Neuroscience Abstracts, (1998) Vol. 24, No. 1-2, pp. 1056.
Meeting Info.: 28th Annual Meeting of the Society for Neuroscience, Part 1 Los Angeles, California, USA November 7-12, 1998 Society for Neuroscience
. ISSN: 0190-5295.
DOCUMENT TYPE: Conference
LANGUAGE: English

L7 ANSWER 28 OF 36 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 1998145713 EMBASE
TITLE: Human gene therapy for hereditary diseases: A review of trials.
AUTHOR: Knoell D.L.; Yiu I.M.
CORPORATE SOURCE: Dr. D.L. Knoell, Div. of Pharm. Practice and Admin., Ohio State University, Parks Hall, 500 W. 12th Avenue, Columbus, OH 43210, United States
SOURCE: American Journal of Health-System Pharmacy, (1 May 1998) 55/9 (899-904).
Refs: 27
ISSN: 1079-2082 CODEN: AHSPEK
COUNTRY: United States
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 022 Human Genetics
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Human gene therapy trials directed at hereditary diseases, including adenosine deaminase (ADA) deficiency, familial hypercholesterolemia, and cystic fibrosis, are reviewed. Human gene therapy involves the introduction and expression of recombinant genes in somatic, nonreproductive cells with the intent to reverse or prevent a particular disease. Two methods for introducing genes into human cells are currently being used in clinical trials. **Ex vivo gene delivery** involves removing targeted cells from the patient's body, introducing the recombinant gene into the cells, and placing the modified cells back into the patient's body. **In vivo gene delivery** involves placing the recombinant gene directly into the patient's body, targeting the tissue or cell of interest. The transfer of the recombinant gene into the cell and the subsequent expression of the transgene product are the rate-limiting steps for successful gene therapy. A variety of methods, including the use of modified viruses and synthetic vectors, are currently being used in clinical trials. Since the approval and initiation of the first human gene therapy trial to treat ADA deficiency in 1989, there have been more than 170 approved gene therapy trials in the United States. More

than 1500 patients have been enrolled in human gene therapy trials worldwide. Preliminary clinical trials have targeted diseases such as ADA deficiency, familial hypercholesterolemia, and cystic fibrosis. These trials have employed variable designs and strategies, making interpretation of the results difficult. However, the initial data are encouraging, and the procedures have been well tolerated. The clinical utility of human gene therapy remains to be defined; immediate efforts will focus on improving vector design to limit toxicity and enhance the efficiency of gene transfer.

L7 ANSWER 29 OF 36 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 1998256729 EMBASE
TITLE: [Cutaneous gene therapy - Perspectives for gene transfer into keratinocytes].
HAUTGENTHERAPIE - PERSPEKTIVEN DES GENTRANSFERS IN KERATINOZYTEN.
AUTHOR: Braun-Falco M.; Hallek M.
CORPORATE SOURCE: M. Braun-Falco, Genzentrum Ludwig-Maximilians-Univ., Feodor-Lynen Strasse 25, D-81377 Munchen, Germany
SOURCE: Hautarzt, (1998) 49/7 (536-544).
Refs: 55
ISSN: 0017-8470 CODEN: HAUTAW
COUNTRY: Germany
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 013 Dermatology and Venereology
022 Human Genetics
LANGUAGE: German
SUMMARY LANGUAGE: English; German
AB The skin is an attractive target tissue for gene therapy. Because of its accessibility, it is suitable for *in vivo* as well as for **ex vivo gene delivery**. The ability to monitor the treatment site is an advantage for safety considerations. The epidermis is a self-renewing tissue, which provides the possibility of achieving long-term treatment through gene transfer into keratinocyte stem cells. This opens new perspectives for the treatment of dermatological and even systemic diseases. The possibility for corrective intervention in disorders with inherited gene defects appears particularly attractive.

L7 ANSWER 30 OF 36 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1997:89804 BIOSIS
DOCUMENT NUMBER: PREV199799389007
TITLE: Genetics and gene therapy for gastrointestinal diseases.
AUTHOR(S): Chowdhury, Jayanta Roy (1); Chowdhury, Namit Roy; Wu, George Y.
CORPORATE SOURCE: (1) Dep. Med. Molecular Genetics, Seaver Inst. Human Genetics, Albert Einstein Coll. Med., 1300 Morris Park Ave., Bronx, NY 10461-1924 USA
SOURCE: Friedman, G. [Editor]; Jacobson, E. D. [Editor]; McCallum, R. W. [Editor]. (1997) pp. 669-676. Gastrointestinal pharmacology and therapeutics.
Publisher: Lippincott-Raven Publishers 227 East Washington Square, Philadelphia, Pennsylvania 19106, USA.
ISBN: 0-397-51625-8.
DOCUMENT TYPE: Book
LANGUAGE: English

L7 ANSWER 31 OF 36 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 97313945 EMBASE
DOCUMENT NUMBER: 1997313945
TITLE: Direct retrovirus-mediated gene transfer to the synovium of the rabbit knee: Implications for arthritis gene therapy.
AUTHOR: Ghivizzani S.C.; Lechman E.R.; Tio C.; Mule K.M.; Chada S.; McCormack J.E.; Evans C.H.; Robbins P.D.

CORPORATE SOURCE: P.D. Robbins, Dept. Molecular Genetics Biochem., Biomedical Sciences Tower, Univ. Pittsburgh School of Medicine, Pittsburgh, PA 15261, United States
SOURCE: Gene Therapy, (1997) 4/9 (977-982).
Refs: 26
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
031 Arthritis and Rheumatism
LANGUAGE: English
SUMMARY LANGUAGE: English

AB We have investigated the feasibility of using high-titer murine leukemia virus-based retroviral vectors to deliver exogenous genes to naive and chronically inflamed knee joints of rabbits *in vivo*. Intraarticular injection of retrovirus encoding β -galactosidase (β -gal or lacZ) was found to transduce synoviocytes in both naive and inflamed joints, but a significantly higher number of lacZ+ cells were found in inflamed knees. Using a retrovirus encoding a secretable marker, human growth hormone (hGH), quantitative comparison of **ex vivo gene delivery** methods demonstrated that transgene expression following *in vivo* gene transfer was at least equivalent to that of the *ex vivo* method in inflamed knees. In addition, hGH transgene expression was maintained for at least 4 weeks. These experiments suggest that high-titer retroviral vector could be used for efficient *in vivo* gene transfer to inflamed joints in patients with rheumatoid arthritis (RA).

L7 ANSWER 32 OF 36 CAPLUS COPYRIGHT 2003 ACS on STNDUPPLICATE 12
ACCESSION NUMBER: 1996:353783 CAPLUS
DOCUMENT NUMBER: 125:1161
TITLE: **Ex vivo gene delivery** of platelet-derived growth factor increases O-2A progenitors in adult rat spinal cord
AUTHOR(S): Ijichi, A.; Noel, F.; Sakuma, S.; Weil, M. M.; Tofilon, P. J.
CORPORATE SOURCE: Department Experimental Radiotherapy, University Texas M. D. Anderson Cancer Center, Houston, TX, 77030, USA
SOURCE: Gene Therapy (1996), 3(5), 389-395
CODEN: GETHEC; ISSN: 0969-7128
PUBLISHER: Stockton
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The O-2A progenitor cell, which serves as a stem cell for the myelinating oligodendrocyte, has been implicated as a major target for radiation-induced spinal cord injury. In an attempt to increase the no. of O-2A cells in the spinal cord, we applied an *ex vivo* gene therapy procedure for delivering platelet-derived growth factor (PDGF). Recombinant fibroblasts expressing PDGF A chain were injected into the cisterna magna of adult rats, which resulted in cell seeding of the subarachnoid space of the cervical spinal cord. The no. of O-2A progenitors in the cervical spinal cord was then assessed with an *in vitro* clonogenic assay. O-2A cells were found to be increased 8 days after recombinant cell injection, and they remained elevated up to at least 14 days. Anal. of O-2A colonies indicated that the implantation of PDGF-expressing cells increased the no. of O-2A progenitors without affecting their *in vitro* proliferation potential or differentiation capacity. These data suggest that implantation of PDGF-expressing cells in the subarachnoid space of the cervical spinal cord may influence a stem cell population crit. to the repair of demyelinated lesions.

L7 ANSWER 33 OF 36 CAPLUS COPYRIGHT 2003 ACS on STNDUPPLICATE 13
ACCESSION NUMBER: 1996:249829 CAPLUS
DOCUMENT NUMBER: 124:308021

TITLE: Long-term expression of human growth hormone (hGH) in mice containing allogeneic yolk sac cell derived neovascular implants expressing hGH

AUTHOR(S): Wei, Yanzhang; Li, Jinhua; Wagner, Thomas E.

CORPORATE SOURCE: Edison Biotechnology Institute, Ohio University, Athens, OH, USA

SOURCE: Stem Cells (Dayton) (1996), 14(2), 232-8

CODEN: STCEEJ; ISSN: 1066-5099

PUBLISHER: AlphaMed Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB We have established a systemic gene delivery animal model system by using cultured murine embryonic yolk sac cells, which can be easily genetically modified in vitro and participate in angiogenesis in vivo when basement membrane proteins (Matrigel) are provided in syngeneic mice. In the present study, we successfully applied this system to allogeneic mice. To suppress donor cell-specific immune responses, the costimulatory signal transduction pathway of T cell activation was blocked by treating the recipient allogeneic C57BL/6 mice with rat-antimouse B7.2 antibody. As a result of this suppression, human growth hormone, the therapeutic gene product, could be detected for over 340 days, while it could only be detected in mice treated with rat-IgG2a, the isotype control of anti-B7.2, for fewer than 50 days. This is the first **ex vivo gene delivery** system that can express a therapeutic gene product, long-term, in an allogeneic host.

L7 ANSWER 34 OF 36 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 14

ACCESSION NUMBER: 1996:271600 BIOSIS

DOCUMENT NUMBER: PREV199698827729

TITLE: Liver-directed gene transfer and application to therapy.

AUTHOR(S): Sandig, V.; Strauss, M. (1)

CORPORATE SOURCE: (1) Max-Planck-Gesellschaft, Humboldt Univ., Max Delbrück Cent. Mol. Med., MPG Gruppe Robert-Rosse-Strasse 10, D-13122 Berlin-Buch Germany

SOURCE: Journal of Molecular Medicine (Berlin), (1996) Vol. 74, No. 4, pp. 205-212.

ISSN: 0946-2716.

DOCUMENT TYPE: General Review

LANGUAGE: English

AB The liver is an important and attractive target for the development of gene therapy strategies. Many genetic diseases are manifested in the liver, and both infectious and malignant diseases affect this organ. Retroviral and adenoviral vectors have been shown to infect hepatocytes with varying, efficiency in vitro and in vivo. The presence of unique receptors at the cellular membrane of hepatocytes has stimulated the development of transfer strategies based on receptor targeting of vectors. The results of a first clinical trial for gene therapy in the liver based on **ex vivo gene delivery** has shown both the feasibility and the limits of current technology. This review discusses both existing vectors and strategies and prospective developments towards liver-directed gene therapy of genetic and malignant diseases.

L7 ANSWER 35 OF 36 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 1995:514894 BIOSIS

DOCUMENT NUMBER: PREV199598529194

TITLE: Modulation of O-2A progenitor cells in the rat cervical spinal cord by **ex vivo gene delivery** of platelet derived growth factor.

AUTHOR(S): Noel, F.; Ichiji, A.; Sakuma, S.; Weil, M.; Tofilon, P. J.

CORPORATE SOURCE: Dep. Experimental Radiotherapy, MD Anderson Cancer Cent., Houston, TX 77030 USA

SOURCE: Society for Neuroscience Abstracts, (1995) Vol. 21, No. 1-3, pp. 1561.
Meeting Info.: 25th Annual Meeting of the Society for Neuroscience San Diego, California, USA November 11-16, 1995
ISSN: 0190-5295.
DOCUMENT TYPE: Conference
LANGUAGE: English

L7 ANSWER 36 OF 36 CAPLUS COPYRIGHT 2003 ACS on STNDUPPLICATE 15
ACCESSION NUMBER: 1994:143866 CAPLUS
DOCUMENT NUMBER: 120:143866
TITLE: Retrovirus-mediated gene transfer into canine thyroid using an ex vivo strategy
AUTHOR(S): O'Malley, Bert W. Jr.; Adams, R. Mark; Sikes, Michael L.; Sawada, Takako; Ledley, Fred D.
CORPORATE SOURCE: Dep. Cell Biol., Baylor Coll. Med., Houston, TX, 77030, USA
SOURCE: Human Gene Therapy (1993), 4(2), 171-8
CODEN: HGTHE3; ISSN: 1043-0342
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The authors describe studies in a canine model aimed at establishing methods for **ex vivo gene delivery** to thyroid follicular cells. Canine follicular cells were harvested from tissue obtained by unilateral lobectomy, grown in TSH-contg. media, and transduced with amphotropic retroviral vectors carrying Escherichia coli beta.-galactosidase or Tn7 neomycin-resistance genes. Up to 30% of cells were transduced with retroviral vectors contg. the neomycin resistance gene, and transduced cells could be selected with G418. Significantly, transduced and selected cells exhibited the morphol. of thyroid follicular cells and continued to express thyroglobulin. To assess the viability of cultivated and transduced cells for transplantation, cells were stained with the vital fluorescent dye DiI, recovered by trypsinization, and transplanted into the contralateral thyroid lobe of autologous animals. Engraftment was demonstrated by fluorescence microscopy and identification of proviral sequences 7-10 days after transplantation. Proviral transcripts were evident using coupled reverse transcription and the polymerase chain reaction using total RNA from transplanted glands. Thyroid follicular cells may represent an attractive target for gene therapy due to their proliferative potential, their large protein synthetic and secretory capacity, and their susceptibility to regulation. The thyroid might be a target for therapy of congenital or acquired thyroid diseases as well as disorders requiring regulated expression of proteins in the circulation. This work demonstrates the feasibility of **ex vivo gene delivery** to thyroid follicular cells that may be used in future investigations.

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L9 ANSWER 1 OF 33 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:465615 CAPLUS
TITLE: Transplantation of human adult astrocytes: Efficiency and safety requirements for an autologous gene therapy
AUTHOR(S): Ridet, Jean-Luc; Sarkis, Chamsy; Serguera, Che; Zennou, Veronique; Charneau, Pierre; Mallet, Jacques
CORPORATE SOURCE: CNRS UMR 9923, Laboratoire de Genetique Moleculaire de la Neurotransmission et des Processus Neurodegeneratifs, bat. CERVI, Hopital Pitie-Salpetriere, Paris, F-75013, Fr.
SOURCE: Journal of Neuroscience Research (2003), 72(6), 704-708
CODEN: JNREDK; ISSN: 0360-4012
PUBLISHER: Wiley-Liss, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Ex vivo gene therapy** is emerging as a promising approach for the treatment of neurodegenerative diseases and central nervous system (CNS) trauma. We have shown previously that human adult astrocytes can be expanded in vitro and can express various therapeutic transgenes (Ridet et al. [1999] Hum. Gene Ther. 10:271-280; Serguera et al. [2001] Mol. Ther. 3:875-881). Here, we grafted normal and lentivirally-modified human adult astrocytes into the striatum and spinal cord of nude mice to test whether they are suitable candidates for ex vivo CNS gene therapy. Transplanted cells survived for at least 2 mo (longest time analyzed) and sustained transgene expression. Importantly, the absence of proliferating cell nuclear antigen (PCNA) staining, a hallmark of cell division, ascertains the safety of these cells. Thus, adult human astrocytes are a promising tool for human CNS repair; they may make autologous ex vivo gene transfer feasible, thereby avoiding the **problems** of immunol. rejection and the side effects of immunosuppressors.

REFERENCE COUNT: 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 2 OF 33 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:458108 CAPLUS
TITLE: Prospects for the treatment of stroke using gene therapy
AUTHOR(S): Zhao, Heng; Yenari, Midori A.; Sapolsky, Robert M.; Steinberg, Gary K.
CORPORATE SOURCE: Dep. of Neurosurg, Stanford stroke Center, Stanford Univ., Stanford, CA, USA
SOURCE: Expert Review of Neurotherapeutics (2003), 3(3), 357-372
CODEN: ERNXAR; ISSN: 1473-7175
PUBLISHER: Future Drugs Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Recent advances have demonstrated the use of gene therapy in the treatment of stroke in exptl. animal models of focal ischemia, global ischemia and subarachnoid hemorrhage. Several different vectors for gene transfer have been studied including herpes simplex virus, adenovirus, adeno-assocd. virus and liposomes. Genetically modified cell lines (e.g., bone marrow-derived cells) have been studied for **ex vivo gene therapy**. The effects of gene transfer to several brain regions including the striatum, cortex, hippocampus, subarachnoid space and blood vessels are reviewed. Targets of gene therapy, such as mol. cascades after ischemia onset (Ca²⁺ influx, ATP loss, increased nitric oxide) and events assocd. with apoptosis are also reviewed, in addn. to how gene transfer may be used to understand pathomechanisms underlying ischemic injury and the temporal therapeutic windows following

ischemia within which protective effects of gene therapy have been achieved. The prospects for gene therapy for stroke are discussed in light of these findings and it is concluded that solns. to key technol. **problems** will allow gene therapy to be a viable treatment modality.

REFERENCE COUNT: 161 THERE ARE 161 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 3 OF 33 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:662225 CAPLUS
DOCUMENT NUMBER: 138:198036
TITLE: Gene therapy for restenosis: current status
AUTHOR(S): Rutanen, Juha; Markkanen, Johanna; Yla-Herttuala, Seppo
CORPORATE SOURCE: A. I. Virtanen Institute, University of Kuopio, Kuopio, Finland
SOURCE: Drugs (2002), 62(11), 1575-1585
CODEN: DRUGAY; ISSN: 0012-6667
PUBLISHER: Adis International Ltd.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review. Atherosclerosis is a major cause of morbidity and mortality in the Western world. Vascular occlusion caused by atherosclerosis usually requires invasive treatment, such as surgical bypass or angioplasty. However, bypass graft failure and restenosis limit the usefulness of these procedures, with 20% of the patients needing a new revascularization procedure within 6 mo of angioplasty. Numerous pharmacol. agents have been investigated for the prevention of restenosis but none has shown undisputed efficacy in clin. medicine. Gene transfer offers a novel approach to the treatment of restenosis because of easy accessibility of vessels and already existing gene delivery methods. It can be used to overexpress therapeutically important proteins locally without high systemic toxicity, and the therapeutic effect can be targeted to a particular pathophysiolog. event. Promising results have been obtained from many pre-clin. expts. using therapeutic genes or oligonucleotides to prevent restenosis. Early clin. trials have shown that plasmid- and adenovirus-mediated vascular gene transfers can be conducted safely and are well tolerated. **Ex vivo gene therapy** with E2F-decoy succeeded in reducing graft occlusion rate after surgical bypass in a randomized, double-blind clin. trial. In the future, further development of gene delivery methods and vectors is needed to improve the efficacy and safety of gene therapy. Also, better knowledge of vascular biol. at the mol. level is needed to find optimal strategies and gene combinations to treat restenosis. Provided that these **difficulties** can be solved, gene therapy offers an enormous potential for clin. medicine in the future.

REFERENCE COUNT: 77 THERE ARE 77 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 4 OF 33 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:659293 CAPLUS
DOCUMENT NUMBER: 138:215194
TITLE: Feasibility of **ex vivo gene therapy** for neurological disorders using the new retroviral vector GCDNsap
AUTHOR(S): Suzuki, A.; Obi, K.; Urabe, T.; Hayakawa, H.; Yamada, M.; Kaneko, S.; Onodera, M.; Mizuno, Y.; Mochizuki, H.
CORPORATE SOURCE: Department of Neurology, Juntendo University School of Medicine, Tokyo, 113-8421, Japan
SOURCE: Journal of Neurochemistry (2002), 82(4), 953-960
CODEN: JONRA9; ISSN: 0022-3042

PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Neuronal progenitor cells (NPC) are particularly suited as the target population for genetic and cellular therapy of neurol. disorders such as Parkinson's disease or stroke. However, genetic modification of these cells using retroviral vectors remains a great challenge because of the low transduction rate and the need for fetal calf serum (FCS) during the transduction process that induces the cell differentiation to mature neurons. To overcome these **problems**, we developed a new retrovirus prodn. system in which the simplified retroviral vector GCDNsap engineered to be resistant to de novo methylation was packaged in the vesicular stomatitis virus G protein (VSV-G), concd. by centrifugation, and resuspended in serum-free medium (StemPro-34 SFM). In transduction expts. using enhanced green fluorescent protein (EGFP) as a marker, the concd. FCS-free virus supernatant infected NPC at a high rate, while maintaining the ability of these cells to self-renew and differentiate in vitro. When such cells were grafted into mouse brains, EGFP-expressing NPC were detected in the region around the injection site at 8 wk post transplantation. These findings suggest that the gene transfer system described here may provide a useful tool to genetically modify NPC for treatments of neurol. disorders.

REFERENCE COUNT: 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 5 OF 33 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:196183 CAPLUS
DOCUMENT NUMBER: 137:89101
TITLE: A novel minimal-size vector (MIDGE) improves transgene expression in colon carcinoma cells and avoids transfection of undesired DNA
AUTHOR(S): Schakowski, Frank; Gorschluter, Marcus; Junghans, Claas; Schroff, Matthias; Buttgereit, Peter; Ziske, Carsten; Schottker, Bjorn; Konig-Merediz, Sven A.; Sauerbruch, Tilman; Wittig, Burghardt; Schmidt-Wolf, Ingo G. H.
CORPORATE SOURCE: Department of Internal Medicine I, University of Bonn, Bonn, 53105, Germany
SOURCE: Molecular Therapy (2001), 3(5, Pt. 1), 793-800
CODEN: MTOHCK; ISSN: 1525-0016
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Viral and plasmid vectors may cause unwanted immunol. side effects resulting from the expression of nontherapeutic genes contained in their sequence. Furthermore, replication-defective viral vectors carry the potential risk of recombination with wild-type viruses or activation of oncogenes. A new vector type for minimalistic, immunol. defined gene expression (MIDGE) may overcome these **problems**. MIDGE is a minimal-size gene transfer unit contg. the expression cassette, including promoter, gene, and RNA-stabilizing sequence, flanked by two short hairpin oligonucleotide sequences. The resulting vector is a small, linear, covalently closed, dumb-bell-shaped mol. DNA not encoding the desired gene is reduced to a min. Here, the authors transfected colon carcinoma cell lines using cationic lipid, cationic polymer, and electroporation with several MIDGE vectors and corresponding plasmids contg. transgenes encoding enhanced green fluorescent protein (eGFP) and human interleukin-2 (hIL-2). Transfection efficiency as measured qual. and quant. with eGFP was found to be comparable for both vector types. However, hIL-2 secretion and eGFP expression were approx. two- to fourfold higher in most cells transfected with these transgenes using MIDGE vectors compared to the plasmid control. This report demonstrates the advantages of this new vector type and its prospects for **ex vivo** gene

therapy studies. (c) 2001 Academic Press.

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 6 OF 33 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:648698 CAPLUS
DOCUMENT NUMBER: 136:318619
TITLE: Liver-directed gene therapy: 2000 and beyond
AUTHOR(S): Lee, S. J.; Takahashi, M.; Kawashita, Y.; Kadakol, A.; Ghosh, S. S.; Parashar, B.; Chowdhury, N. Roy; Chowdhury, J. Roy

CORPORATE SOURCE:

SOURCE: Falk Symposium (2001), 115(Liver Cirrhosis and Its Development), 253-262
CODEN: FASYDI; ISSN: 0161-5580

PUBLISHER: Kluwer Academic Publishers

DOCUMENT TYPE: Journal; General Review

LANGUAGE: English

AB A review which discusses the current goals, methods, and challenges of gene therapy for liver diseases in the new millennium. Topics discussed include gene transfer to the liver using non-viral vehicles; recombinant viruses as gene delivery vehicles; site-directed gene repair; and **ex vivo gene therapy**.

REFERENCE COUNT: 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 7 OF 33 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:382062 CAPLUS
DOCUMENT NUMBER: 135:277815
TITLE: Encapsulated cell implants as a novel treatment for Parkinson's disease
AUTHOR(S): Tseng, Jack L.; Aebischer, Patrick
CORPORATE SOURCE: Division of Surgical Research and Gene Therapy Center, Lausanne University Medical School, Lausanne, Switz.
SOURCE: Methods in Molecular Medicine (2001), 62(Parkinson's Disease), 279-288
CODEN: MMMEFN

PUBLISHER: Humana Press Inc.

DOCUMENT TYPE: Journal; General Review

LANGUAGE: English

AB A review with 47 refs. Topics discussed include **in vivo** and **ex vivo gene therapy**; polymer encapsulation of genetically engineered cells; major **problems** with delivering neurotropic factors **in vivo**; and materials and methods for prep. cells for implantation.

REFERENCE COUNT: 47 THERE ARE 47 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 8 OF 33 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:119424 CAPLUS
DOCUMENT NUMBER: 135:116298
TITLE: Adrenocortical cells immortalized by telomerase: Potential use for **ex vivo gene therapy**
AUTHOR(S): Hornsby, Peter J.; Ozol, Khan; Yang, Keyi
CORPORATE SOURCE: Huffington Center on Aging, Baylor College of Medicine, Houston, TX, USA
SOURCE: Journal of Anti-Aging Medicine (2000), 3(4), 411-417
CODEN: JAMEF8; ISSN: 1094-5458
PUBLISHER: Mary Ann Liebert, Inc.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review, with 37 refs. Telomerization, the process of immortalization of normal cells by expression of telomerase reverse transcriptase (TERT), could be of great use in biomedicine if the process allows cells to retain their normal properties and does not promote neoplastic transformation. In this article, we review the data on the potential uses of telomerized cells in **ex vivo gene therapy**, and discuss the issue of the potential risks of the use of this technol. We present preliminary data on the transplantation of telomerized bovine adrenocortical cells in the rat brain. Like other cell types, adrenocortical cells may be engineered to secrete desirable gene products. Currently, **problems** of immune rejection limit the usefulness of this potential therapy. We discuss future improvements in this cell transplantation system that could address these questions. Telomerization, by removing the senescence barrier to unlimited cell proliferation, will greatly aid the genetic modification of cells in order to solve the issue of immune rejection and other **problems**.

REFERENCE COUNT: 37 THERE ARE 37 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 9 OF 33 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1996:88056 CAPLUS
DOCUMENT NUMBER: 124:185243
TITLE: Toward development of an implantable tissue engineered liver
AUTHOR(S): Davis, Matthew W.; Vacanti, Joseph P.
CORPORATE SOURCE: Dep. Chemical Engineering, Massachusetts Inst. Technology, Cambridge, MA, 02139, USA
SOURCE: Biomaterials (1996), 17(3), 365-72
CODEN: BIMADU; ISSN: 0142-9612
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review, with 74 refs. Hepatocyte transplantation on implantable devices is a tissue engineering approach to improve the treatment of liver disease and the efficacy of **ex vivo gene therapy**. Diverse physiol. functions and high metabolic activity of the liver represent significant **challenges** to engineering implantable devices that provide long-term hepatic support. Liver tissue engineering research has explored alternatives to direct hepatocyte injection that include hepatocyte attachment to microcarriers, encapsulation and transplantation on biodegradable polymer scaffolds. Successful function of hepatocytes transplanted on implantable devices in animal models has been documented by prodn. of albumin and other liver-specific markers, and clearance of bilirubin and urea metabolites. Strategies used to achieve these successes are discussed, with particular emphasis on biodegradable polymer scaffolds, and two areas of investigation that may improve the function of implantable tissue engineered liver devices are highlighted.

L9 ANSWER 10 OF 33 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1995:533457 CAPLUS
DOCUMENT NUMBER: 123:134272
TITLE: Ex vivo hepatic gene transfer in mouse using a defective herpes simplex virus-1 vector
AUTHOR(S): Lu, Bing; Gupta, Sanjeev; Federoff, Howard
CORPORATE SOURCE: Departments Molecular Genetics, Albert Einstein College Medicine, Bronx, NY, 10461, USA
SOURCE: Hepatology (Philadelphia, PA, United States) (1995), 21(3), 752-9
CODEN: HPTLD9; ISSN: 0270-9139
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A defective amplicon herpes simplex virus-1 (HSV-1) vector, HSVlac, was

used to transfer an *E. coli* lacZ reporter gene into primary hepatocytes. The lacZ gene was driven by the HSV immediate early (IE) 4/5 promoter. Use of the HSVlac vector resulted in highly efficient gene transfer. Because **difficulties** in culturing primary hepatocytes impose limitations in **ex vivo gene therapy**

, the authors sought to det. whether use of the HSVlac vector could simplify gene transfer. Therefore, the authors incubated HSVlac with primary hepatocytes in suspension and found that the lacZ gene was still transferred with great rapidity and efficiency. To examine lacZ expression in transduced hepatocytes *in vivo*, the authors used a mouse hepatocyte transplantation system. In congenetic recipients of primary hepatocytes transduced with HSVlac in suspension, the lacZ gene was expressed in liver and spleen up to 2 wk. However, survival of transplanted hepatocytes, as well as persistence of HSVlac genome in recipient organs, was demonstrated for up to an 11-wk duration of the expt. These findings suggest that *in vivo* regulation of the HSV IE4/5 promoter was responsible for the short-term expression of lacZ, which should be overcome by the use of liver-specific promoters. Therefore, the results indicate the feasibility of hepatic gene transfer with a defective HSV-1 vector.

L9 ANSWER 11 OF 33 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2003:335018 BIOSIS
DOCUMENT NUMBER: PREV200300335018
TITLE: A highly efficient, stable, and rapid approach for *ex vivo* human liver gene therapy via a FLAP lentiviral vector.
AUTHOR(S): Giannini, Carlo (1); Morosan, Serban; Tralhao, J.
Guilherme; Guidotti, Jacques Emmanuel; Battaglia, Serena;
Mollier, Karine; Hannoun, Laurent; Kremsdorff, Dina;
Gilgenkrantz, Helene; Charneau, Pierre
CORPORATE SOURCE: (1) PASTEUR INSERM Unite 370, Necker Institute, 156, Rue de Vaugirard, 75015, Paris, France: giannini@pasteur.fr France
SOURCE: Hepatology, (July 2003, 2003) Vol. 38, No. 1, pp. 114-122.
print.
ISSN: 0270-9139.

DOCUMENT TYPE: Article
LANGUAGE: English

AB Allogenic hepatocyte transplantation or autologous transplantation of genetically modified hepatocytes has been used successfully to correct congenital or acquired liver diseases and can be considered as an alternative to orthotopic liver transplantation. However, hepatocytes are neither easily maintained in culture nor efficiently genetically modified and are very sensitive to dissociation before their reimplantation into the recipient. These **difficulties** have greatly limited the use of an *ex vivo* approach in clinical trials. In the present study, we have shown that primary human and rat hepatocytes can be efficiently transduced with a FLAP lentiviral vector without the need for plating and culture. Efficient transduction of nonadherent primary hepatocytes was achieved with a short period of contact with vector particles, without modifying hepatocyte viability, and using reduced amounts of vector. We also showed that the presence of the DNA FLAP in the vector construct was essential to reach high levels of transduction. Moreover, transplanted into uPA/SCID mouse liver, lentivirally transduced primary human hepatocytes extensively repopulated their liver and maintained a differentiated and functional phenotype as assessed by the stable detection of human albumin and antitrypsin in the serum of the animals for months. In conclusion, the use of FLAP lentiviral vectors allows, in a short period of time, a high transduction efficiency of human functional and reimplantable hepatocytes. This work therefore opens new perspectives for the development of human clinical trials based on liver-directed **ex vivo gene therapy**.

L9 ANSWER 12 OF 33 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2003:325664 BIOSIS
DOCUMENT NUMBER: PREV200300325664
TITLE: TRANSGENE EXPRESSION AND CELL SORTING OF RAT NEURAL PROGENITOR CELLS INFECTED WITH A LENTIVIRAL VECTOR.
AUTHOR(S): Duan, W. M. (1); Halter, J. (1); Kessler, J. A.; Bohn, M. C. (1)
CORPORATE SOURCE: (1) CMIER Neurobiology Program, Northwestern Univ's Feinberg Sch of Med, Chicago, IL, USA USA
SOURCE: Society for Neuroscience Abstract Viewer and Itinerary Planner, (2002) Vol. 2002, pp. Abstract No. 691.12. <http://sfn.scholarone.com>. cd-rom.
Meeting Info.: 32nd Annual Meeting of the Society for Neuroscience Orlando, Florida, USA November 02-07, 2002
Society for Neuroscience

DOCUMENT TYPE: Conference
LANGUAGE: English

AB Poor transduction efficiency associated with transient, low transgene expression levels remains one of major **obstacles** toward the use of viral vectors for gene therapy. The aim of present study was to explore a novel cellular gene delivery system in which neural progenitor cells are used as gene delivery vehicles. Neural progenitor cells were prepared from the subventricular zoon of Fischer 344 neonatal rats (P1-2). Briefly, striatal tissue from the forebrain was dissected and dissociated. Around 50,000 cells were plated into 10 cm petridishes containing serum-free N2 medium supplemented with bFGF (10ng/ml). Neurospheres that had formed by 4-5 days post-plating were triturated and then infected with a self-inactivating HIV-1-based lentiviral vector encoding enhanced green fluorescent protein (eGFP). The transduction resulted in infection of >70% undifferentiated neural progenitor cells and long-term expression of eGFP (4 weeks). To purify infected cells, fluorescence activated-cell sorting was conducted. After cell sorting, 99% cells were eGFP positive. They were plated into poly-D-lysine pre-coated culture dishes and studied for differentiated phenotype. Our preliminary results show that the transduction of neural progenitor cells with lentiviral vectors leads to robust and stable gene expression in vitro and that infected cells can be sorted by cell sorting. This approach may be useful for **ex vivo gene therapy**.

L9 ANSWER 13 OF 33 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:505788 BIOSIS

DOCUMENT NUMBER: PREV200200505788

TITLE: Feasibility of **ex vivo gene therapy** for neurological disorders using the new retroviral vector GCDNsap packaged in the vesicular stomatitis virus G protein.

AUTHOR(S): Suzuki, A.; Obi, K.; Urabe, T.; Hayakawa, H.; Yamada, M.; Kaneko, S.; Onodera, M. (1); Mizuno, Y.; Mochizuki, H.

CORPORATE SOURCE: (1) Department of Hematology, Institutes of Clinical Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, 305-8575: hideki@med.juntendo.ac.jp Japan

SOURCE: Journal of Neurochemistry, (August, 2002) Vol. 82, No. 4, pp. 953-960. <http://www.blacksci.co.uk/jnc>. print.
ISSN: 0022-3042.

DOCUMENT TYPE: Article
LANGUAGE: English

AB Neuronal progenitor cells (NPC) are particularly suited as the target population for genetic and cellular therapy of neurological disorders such as Parkinson's disease or stroke. However, genetic modification of these cells using retroviral vectors remains a great challenge because of the low transduction rate and the need for fetal calf serum (FCS) during the transduction process that induces the cell differentiation to mature neurons. To overcome these **problems**, we developed a new

retrovirus production system in which the simplified retroviral vector GCDNsap engineered to be resistant to de novo methylation was packaged in the vesicular stomatitis virus G protein (VSV-G), concentrated by centrifugation, and resuspended in serum-free medium (StemPro-34 SFM). In transduction experiments using enhanced green fluorescent protein (EGFP) as a marker, the concentrated FCS-free virus supernatant infected NPC at a high rate, while maintaining the ability of these cells to self-renew and differentiate in vitro. When such cells were grafted into mouse brains, EGFP-expressing NPC were detected in the region around the injection site at 8 weeks post transplantation. These findings suggest that the gene transfer system described here may provide a useful tool to genetically modify NPC for treatments of neurological disorders.

L9 ANSWER 14 OF 33 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2001:301977 BIOSIS
DOCUMENT NUMBER: PREV200100301977
TITLE: A novel type of minimal size non-viral vector with improved safety properties for clinical trials and enhanced transgene expression: First results.
AUTHOR(S): Schakowski, Frank (1); Buttgereit, Peter (1); Gorschlüter, Marcus (1); Junghans, Claas; Schröff, Matthias; Wittig, Burghardt; Schmidt-Wolf, Ingo G. H. (1)
CORPORATE SOURCE: (1) Dpt. of General Internal Medicine I, Rheinische Friedrich-Wilhelms University, Bonn Germany
SOURCE: Blood, (November 16, 2000) Vol. 96, No. 11 Part 2, pp. 383b. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
ISSN: 0006-4971.
DOCUMENT TYPE: Conference
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Vector systems for cancer gene therapy strategies should offer both a means of successful transfection and a maximum of safety for the patient. Replication defective viral vectors are effective but carry the potential risk of recombination with wild-type viruses or activation of oncogenes. Viral and plasmid vectors may further cause unwanted immunological side effects resulting from the expression of therapeutically unwanted genes contained in their sequence. A new vector type for minimalistic, immunological defined gene expression (MIDGE) may overcome these problems. Here we present construction principle and first transfection data. MIDGE is a minimalistic gene transfer unit containing the expression cassette, including promotor, gene and RNA-stabilizing sequence, flanked by two short hairpin oligonucleotide sequences. The resulting vector is a small linear, covalently closed, dumbbell-shaped molecule. We transfected colon carcinoma cell lines by electroporation and cationic complexes (e.g. branched polyethylenimine, lipofectamine) with MIDGE vectors and corresponding plasmids containing transgenes encoding human interleukin-2 (hIL-2) and enhanced green fluorescent (eGFP) protein. Both transfection methods show no differences in the percentage of transfected cells but the amount of expressed protein was several times higher in cells transfected with the MIDGE vector compared to the plasmid control. Our results demonstrate the utility of this new vector type and its perspectives for **ex vivo gene therapy** studies.

L9 ANSWER 15 OF 33 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2000:116917 BIOSIS
DOCUMENT NUMBER: PREV200000116917
TITLE: Gene therapy for hemophilia.
AUTHOR(S): Hortelano, G.; Chang, P. L. (1)
CORPORATE SOURCE: (1) Department of Pediatrics 3N18, McMaster University,

SOURCE: 1200 Main Street West, Hamilton, Ontario, L8N 3Z5 Canada
Artificial Cells Blood Substitutes and Immobilization
Biotechnology, (Jan., 2000) Vol. 28, No. 1, pp. 1-24.
ISSN: 1073-1199.

DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Hemophilia A and B are X-linked genetic disorders caused by deficiency of the coagulation factors VIII and IX, respectively. Because of the health hazards and costs of current product replacement therapy, much effort is devoted to the development of gene therapy for these disorders. Approaches to gene therapy for the hemophilias include: **ex vivo** gene therapy in which cells from the intended recipients are explanted, genetically modified to secrete Factor VIII or IX, and reimplanted into the donor; **in vivo** gene therapy in which Factor VIII or IX encoding vectors are directly injected into the recipient; and non-autologous gene therapy in which universal cell lines engineered to secrete Factor VIII or IX are enclosed in immuno-protective devices before implantation into recipients. Research into these approaches is aided by the many murine and canine models available. While **problems** of achieving high and sustained levels of factor delivery, and issues related to efficacy, safety and cost are still to be resolved, progress in gene therapy for the hemophilias has been encouraging and is likely to reach human clinical trial in the foreseeable future.

L9 ANSWER 16 OF 33 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1999:400315 BIOSIS
DOCUMENT NUMBER: PREV199900400315
TITLE: Hepatocyte transplantation for the treatment of human disease.
AUTHOR(S): Strom, Stephen C. (1); Chowdhury, Jayanta Roy; Fox, Ira J. (1)
CORPORATE SOURCE: Dep. Pathol., Univ. Pittsb., Pittsburgh, PA 15261 USA
SOURCE: Seminars in Liver Disease, (1999) Vol. 19, No. 1, pp. 39-48.
ISSN: 0272-8087.
DOCUMENT TYPE: General Review
LANGUAGE: English

L9 ANSWER 17 OF 33 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1999:39364 BIOSIS
DOCUMENT NUMBER: PREV199900039364
TITLE: Long-term amelioration of bilirubin glucuronidation defect in Gunn rats by transplanting genetically modified immortalized autologous hepatocytes.
AUTHOR(S): Tada, Kouji; Roy-Chowdhury, Namita (1); Prasad, Vinayaka; Kim, Byung-Ho; Manchikalapudi, P.; Fox, Ira J.; Van Duijvendijk, Peter; Bosma, Piter J.; Roy-Chowdhury, Jayanta (1)
CORPORATE SOURCE: Marion Bessin Liver Research Cent., Albert Einstein Coll. Med., 1300 Morris Park Ave., Bronx, NY 10462 USA
SOURCE: Cell Transplantation, (Nov.-Dec., 1998) Vol. 7, No. 6, pp. 607-616.
ISSN: 0963-6897.
DOCUMENT TYPE: Article
LANGUAGE: English

AB **Ex vivo gene therapy**, in which hepatocytes are harvested from mutants, retrovirally transduced with a normal gene and transplanted back into the donor, has been used for correction of inherited metabolic defects of liver. Major drawbacks of this method include limited availability of autologous hepatocytes, inefficient retroviral transduction of primary hepatocytes, and the limited number of hepatocytes that can be transplanted safely. To obviate these **problems**, we transduced primary hepatocytes derived from inbred bilirubin-UDP-glucuronosyltransferase (UGT)-deficient Gunn rats by

infection with a recombinant retrovirus expressing temperature-sensitive mutant SV40 large T antigen (tsT). The immortalized cells were then transduced with a second recombinant retrovirus expressing human B-UGT, and a clone expressing high levels of the enzyme was expanded by culturing at permissive temperature (33degreeC). At 37degreeC, tsT antigen was degraded and the cells expressed UGT activity toward bilirubin at a level approximately twice that present in normal rat liver homogenates. For seeding the cells into the liver bed, 1 X 10⁷ cells were injected into the spleens of syngeneic Gunn rats five times at 10-day intervals. Excretion of bilirubin glucuronides in bile was demonstrated by HPLC analysis and serum bilirubin levels were reduced by 27 to 52% in 40 days after the first transplantation and remained so throughout the duration of the study (120 days). None of the transplanted Gunn rats or SCID mice transplanted with the immortalized cells developed tumors.

L9 ANSWER 18 OF 33 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1997:82156 BIOSIS
DOCUMENT NUMBER: PREV199799373869
TITLE: Vectors: Shuttle vehicles for gene therapy.
AUTHOR(S): Wilson, J. M.
CORPORATE SOURCE: Inst. Human Gene Therapy, 204 Wistar Inst., 3601 Spruce Street, Philadelphia, PA 19104-4268 USA
SOURCE: Clinical and Experimental Immunology, (1997) Vol. 107, No. SUPPL. 1, pp. 31-32.
ISSN: 0009-9104.
DOCUMENT TYPE: Journal; Article
LANGUAGE: English

AB Gene therapy is being considered for the treatment of various inherited and acquired disorders. The basic premise of this new therapeutic modality is manipulation of gene expression towards a therapeutic end. The early development of the field focused on a technique called **ex vivo gene therapy** in which autologous cells are genetically manipulated in culture prior to transplantation. Recent advances have stimulated the development of in vivo gene therapy approaches based on direct delivery of the therapeutic gene to cells in vivo. The rate-limiting technologies of gene therapy are the gene delivery vehicles, called vectors, used to accomplish gene transfer. The most efficient vectors are based on recombinant versions of viruses with retroviral vectors serving as prototypes. This viral vector system has been exploited in ex vivo approaches of gene therapy in which cultured, dividing cells are transduced with the recombinant virus resulting in integration of the proviral DNA into the chromosomal DNA of the recipient cell. The use of retroviral vectors in gene therapy has been restricted to ex vivo approaches because of **difficulties** in purifying the virion and the requirement that the target cell is dividing at the time of transduction. More recently, vectors based on adenoviruses have been developed for in vivo gene therapy. These viruses can be grown in large quantities and highly purified. Importantly, they efficiently transduce the recombinant genome into non-dividing cells. Applications include in vivo gene delivery to a variety of targets such as muscle, lung, liver and the central nervous system. Clinical trials of in vivo delivery with adenoviruses have been undertaken for the treatment of cystic fibrosis.

L9 ANSWER 19 OF 33 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1996:120446 BIOSIS
DOCUMENT NUMBER: PREV199698692581
TITLE: Toward development of an implantable tissue engineered liver.
AUTHOR(S): Davis, Matthew W.; Vacanti, Joseph P. (1)
CORPORATE SOURCE: (1) Dep. Surg., Harvard Med. Sch. Child. Hosp., 300 Longwood Ave., Boston, MA 02115 USA
SOURCE: Biomaterials, (1996) Vol. 17, No. 3, pp. 365-372.
ISSN: 0142-9612.

DOCUMENT TYPE: General Review

LANGUAGE: English

AB Hepatocyte transplantation on implantable devices is a tissue engineering approach to improve the treatment of liver disease and the efficacy of **ex vivo gene therapy**. Diverse physiological functions and high metabolic activity of the liver represent significant **challenges** to engineering implantable devices that provide long-term hepatic support. Liver tissue engineering research has explored alternatives to direct hepatocyte injection that include hepatocyte attachment to microcarriers, encapsulation and transplantation on biodegradable polymer scaffolds. Successful function of hepatocytes transplanted on implantable devices in animal models has been documented by production of albumin and other liver-specific markers, and clearance of bilirubin and urea metabolites. Strategies used to achieve these successes are reviewed, with particular emphasis on biodegradable polymer scaffolds, and two areas of investigation that may improve the function of implantable tissue engineered liver devices are highlighted.

L9 ANSWER 20 OF 33 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN

ACCESSION NUMBER: 2003259355 EMBASE

TITLE: A highly efficient, stable, and rapid approach for *ex vivo* human liver gene therapy via a FLAP lentiviral vector.

AUTHOR: Giannini C.; Morosan S.; Tralhao J.G.; Guidotti J.E.; Battaglia S.; Mollier K.; Hannoun L.; Kremsdorf D.; Gilgenkrantz H.; Charneau P.

CORPORATE SOURCE: Dr. C. Giannini, PASTEUR INSERM Unite 370, Necker Institute, 156, rue de Vaugirard, 75015 Paris, France.
giannini@pasteur.fr

SOURCE: Hepatology, (1 Jul 2003) 38/1 (114-122).
Refs: 33

ISSN: 0270-9139 CODEN: HPTLD

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 005 General Pathology and Pathological Anatomy
029 Clinical Biochemistry
048 Gastroenterology

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Allogenic hepatocyte transplantation or autologous transplantation of genetically modified hepatocytes has been used successfully to correct congenital or acquired liver diseases and can be considered as an alternative to orthotopic liver transplantation. However, hepatocytes are neither easily maintained in culture nor efficiently genetically modified and are very sensitive to dissociation before their reimplantation into the recipient. These **difficulties** have greatly limited the use of an *ex vivo* approach in clinical trials. In the present study, we have shown that primary human and rat hepatocytes can be efficiently transduced with a FLAP lentiviral vector without the need for plating and culture. Efficient transduction of nonadherent primary hepatocytes was achieved with a short period of contact with vector particles, without modifying hepatocyte viability, and using reduced amounts of vector. We also showed that the presence of the DNA FLAP in the vector construct was essential to reach high levels of transduction. Moreover, transplanted into uPA/SCID mouse liver, lentivirally transduced primary human hepatocytes extensively repopulated their liver and maintained a differentiated and functional phenotype as assessed by the stable detection of human albumin and antitrypsin in the serum of the animals for months. In conclusion, the use of FLAP lentiviral vectors allows, in a short period of time, a high transduction efficiency of human functional and reimplantable hepatocytes. This work therefore opens new perspectives for the development of human clinical trials based on liver-directed **ex vivo gene therapy**.

L9 ANSWER 21 OF 33 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 2003224662 EMBASE
TITLE: Transplantation of human adult astrocytes: Efficiency and safety requirements for an autologous gene therapy.
AUTHOR: Ridet J.-L.; Sarkis C.; Serguera C.; Zennou V.; Charneau P.; Mallet J.
CORPORATE SOURCE: Dr. J. Mallet, CNRS UMR 9923, Lab. Genet. Molec.
Neurotransmiss., Hopital Pitie-Salpetriere, 83 boulevard de l'Hopital, F-75013 Paris, France. mallet@infobiogen.fr
SOURCE: Journal of Neuroscience Research, (15 Jun 2003) 72/6 (704-708).
Refs: 23
ISSN: 0360-4012 CODEN: JNREDK
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 008 Neurology and Neurosurgery
021 Developmental Biology and Teratology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB **Ex vivo gene therapy** is emerging as a promising approach for the treatment of neurodegenerative diseases and central nervous system (CNS) trauma. We have shown previously that human adult astrocytes can be expanded in vitro and can express various therapeutic transgenes (Ridet et al. [1999] Hum. Gene Ther. 10:271 - 280; Serguera et al. [2001] Mol. Ther. 3:875-881). Here, we grafted normal and lentivirally-modified human adult astrocytes into the striatum and spinal cord of nude mice to test whether they are suitable candidates for *ex vivo* CNS gene therapy. Transplanted cells survived for at least 2 months (longest time analyzed) and sustained transgene expression. Importantly, the absence of proliferating cell nuclear antigen (PCNA) staining, a hallmark of cell division, ascertains the safety of these cells. Thus, adult human astrocytes are a promising tool for human CNS repair; they may make autologous *ex vivo* gene transfer feasible, thereby avoiding the **problems** of immunological rejection and the side effects of immunosuppressors. .COPYRGT. 2003 Wiley-Liss, Inc.

L9 ANSWER 22 OF 33 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 2003209785 EMBASE
TITLE: Prospects for the treatment of stroke using gene therapy.
AUTHOR: Zhao H.; Yenari M.A.; Sapolsky R.M.; Steinberg G.K.
CORPORATE SOURCE: Dr. G.K. Steinberg, Stanford University, 300 Pasteur Drive R200, Stanford, CA 94305 5327, United States. gsteinberg@stanford.edu
SOURCE: Expert Review of Neurotherapeutics, (2003) 3/3 (357-372).
Refs: 156
ISSN: 1473-7175 CODEN: ERNXAR
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 008 Neurology and Neurosurgery
022 Human Genetics
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Recent advances have demonstrated the use of gene therapy in the treatment of stroke in experimental animal models of focal ischemia, global ischemia and subarachnoid hemorrhage. Several different vectors for gene transfer have been studied including herpes simplex virus, adenovirus, adeno-associated virus and liposomes. Genetically modified cell lines (e.g., bone marrow-derived cells) have been studied for **ex vivo gene therapy**. The effects of gene transfer to several brain regions including the striatum, cortex, hippocampus, subarachnoid space and blood vessels are reviewed. Targets of gene therapy, such as molecular cascades after ischemia onset (Ca(2+)

influx, ATP loss, increased nitric oxide) and events associated with apoptosis are also reviewed, in addition to how gene transfer may be used to understand pathomechanisms underlying ischemic injury and the temporal therapeutic windows following ischemia within which protective effects of gene therapy have been achieved. The prospects for gene therapy for stroke are discussed in light of these findings and it is concluded that solutions to key technological **problems** will allow gene therapy to be a viable treatment modality.

L9 ANSWER 23 OF 33 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 2002296162 EMBASE
TITLE: Feasibility of **ex vivo gene therapy** for neurological disorders using the new retroviral vector GCDNsap packaged in the vesicular stomatitis virus G protein.
AUTHOR: Suzuki A.; Obi K.; Urabe T.; Hayakawa H.; Yamada M.; Kaneko S.; Onodera M.; Mizuno Y.; Mochizuki H.
CORPORATE SOURCE: M. Onodera, Department of Hematology, Institutes of Clinical Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8575, Japan. hideki@med.juntendo.ac.jp
SOURCE: Journal of Neurochemistry, (2002) 82/4 (953-960).
Refs: 18
ISSN: 0022-3042 CODEN: JONRA
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 008 Neurology and Neurosurgery
022 Human Genetics
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Neuronal progenitor cells (NPC) are particularly suited as the target population for genetic and cellular therapy of neurological disorders such as Parkinson's disease or stroke. However, genetic modification of these cells using retroviral vectors remains a great challenge because of the low transduction rate and the need for fetal calf serum (FCS) during the transduction process that induces the cell differentiation to mature neurons. To overcome these **problems**, we developed a new retrovirus production system in which the simplified retroviral vector GCDNsap engineered to be resistant to de novo methylation was packaged in the vesicular stomatitis virus G protein (VSV-G), concentrated by centrifugation, and resuspended in serum-free medium (StemPro-34 SFM). In transduction experiments using enhanced green fluorescent protein (EGFP) as a marker, the concentrated FCS-free virus supernatant infected NPC at a high rate, while maintaining the ability of these cells to self-renew and differentiate in vitro. When such cells were grafted into mouse brains, EGFP-expressing NPC were detected in the region around the injection site at 8 weeks post transplantation. These findings suggest that the gene transfer system described here may provide a useful tool to genetically modify NPC for treatments of neurological disorders.

L9 ANSWER 24 OF 33 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 2002281218 EMBASE
TITLE: Gene therapy for restenosis current status.
AUTHOR: Rutanen J.; Markkanen J.; Yla-Herttuala S.
CORPORATE SOURCE: Dr. S. Yla-Herttuala, Department of Molecular Medicine, A. I. Virtanen Institute, University of Kuopio, P.O. Box 1627, Kuopio, FIN-70211, Finland. Seppo.Ylaherttuala@uku.fi
SOURCE: Drugs, (2002) 62/11 (1575-1585).
Refs: 77
ISSN: 0012-6667 CODEN: DRUGAY
COUNTRY: New Zealand
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 022 Human Genetics

030 Pharmacology
037 Drug Literature Index

LANGUAGE: English
SUMMARY LANGUAGE: English

AB Atherosclerosis is a major cause of morbidity and mortality in Western world. Vascular occlusion caused by atherosclerosis usually requires invasive treatment, such as surgical bypass or angioplasty. However, bypass graft failure and restenosis limit the usefulness of these procedures, with 20% of patients needing a new revascularisation procedure within 6 months of angioplasty. Numerous pharmacological agents have been investigated for the prevention of restenosis but none has shown undisputed efficacy in clinical medicine. Gene transfer offers a novel approach to the treatment of restenosis because of easy accessibility of vessels and already existing gene delivery methods. It can be used to overexpress therapeutically important proteins locally without high systemic toxicity, and the therapeutic effect can be targeted to a particular pathophysiological event. Promising results have been obtained from many pre-clinical experiments using therapeutic genes or oligonucleotides to prevent restenosis. Early clinical trials have shown that plasmid- and adenovirus-mediated vascular gene transfers can be conducted safely and are well tolerated. **Ex vivo** gene therapy with E2F-decoy succeeded in reducing graft occlusion rate after surgical bypass in a randomised, double-blind clinical trial. In the future, further development of gene delivery methods and vectors is needed to improve the efficacy and safety of gene therapy. Also, better knowledge of vascular biology at the molecular level is needed to find optimal strategies and gene combinations to treat restenosis. Provided that these **difficulties** can be solved, gene therapy offers an enormous potential for clinical medicine in the future.

L9 ANSWER 25 OF 33 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN

ACCESSION NUMBER: 2001200408 EMBASE

TITLE: A novel minimal-size vector (MIDGE) improves transgene expression in colon carcinoma cells and avoids transfection of undesired DNA.

AUTHOR: Schakowski F.; Gorschluter M.; Junghans C.; Schroff M.; Buttgereit P.; Ziske C.; Schottker B.; Konig-Merediz S.A.; Sauerbruch T.; Wittig B.; Schmidt-Wolf I.G.H.

CORPORATE SOURCE: I.G.H. Schmidt-Wolf, Department of Internal Medicine 1, University of Bonn, 53105 Bonn, Germany.

SOURCE: picasso@uni-bonn.de
Molecular Therapy, (2001) 3/5 I (793-800).

Refs: 27

ISSN: 1525-0016 CODEN: MTOHCK

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 016 Cancer
022 Human Genetics
029 Clinical Biochemistry
048 Gastroenterology

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Viral and plasmid vectors may cause unwanted immunological side effects resulting from the expression of nontherapeutic genes contained in their sequence. Furthermore, replication-defective viral vectors carry the potential risk of recombination with wild-type viruses or activation of oncogenes. A new vector type for minimalist, immunologically defined gene expression (MIDGE) may overcome these **problems**. MIDGE is a minimal-size gene transfer unit containing the expression cassette, including promoter, gene, and RNA-stabilizing sequence, flanked by two short hairpin oligonucleotide sequences. The resulting vector is a small, linear, covalently closed, dumb-bell-shaped molecule. DNA not encoding the desired gene is reduced to a minimum. Here, we transfected colon carcinoma

cell lines using cationic lipid, cationic polymer, and electroporation with several MIDGE vectors and corresponding plasmids containing transgenes encoding enhanced green fluorescent protein (eGFP) and human interleukin-2 (hIL-2). Transfection efficiency as measured qualitatively and quantitatively with eGFP was found to be comparable for both vector types. However, hIL-2 secretion and eGFP expression were approximately two- to fourfold higher in most cells transfected with these transgenes using MIDGE vectors compared to the plasmid control. This report demonstrates the advantages of this new vector type and its prospects for **ex vivo gene therapy** studies.

L9 ANSWER 26 OF 33 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 2001047956 EMBASE
TITLE: Adrenocortical cells immortalized by telomerase: Potential use for **ex vivo gene therapy**.
AUTHOR: Hornsby P.J.; Ozol K.; Yang K.
CORPORATE SOURCE: Dr. P.J. Hornsby, Huffington Center on Aging, Baylor College of Medicine, 1 Baylor Plaza M320, Houston, TX 77030, United States
SOURCE: Journal of Anti-Aging Medicine, (2000) 3/4 (411-417).
Refs: 37
ISSN: 1094-5458 CODEN: JAMEF8
COUNTRY: United States
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 003 Endocrinology
022 Human Genetics
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Telomerization, the process of immortalization of normal cells by expression of telomerase reverse transcriptase (TERT), could be of great use in biomedicine if the process allows cells to retain their normal properties and does not promote neoplastic transformation. In this article, we review the data on the potential uses of telomerized cells in **ex vivo gene therapy**, and discuss the issue of the potential risks of the use of this technology. We present preliminary data on the transplantation of telomerized bovine adrenocortical cells in the rat brain. Like other cell types, adrenocortical cells may be engineered to secrete desirable gene products. Currently, **problems** of immune rejection limit the usefulness of this potential therapy. We discuss future improvements in this cell transplantation system that could address these questions. Telomerization, by removing the senescence barrier to unlimited cell proliferation, will greatly aid the genetic modification of cells in order to solve the issue of immune rejection and other **problems**.

L9 ANSWER 27 OF 33 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 2000053704 EMBASE
TITLE: Gene therapy for hemophilia.
AUTHOR: Hortelano G.; Chang P.L.
CORPORATE SOURCE: Dr. P.L. Chang, Department of Pediatrics, McMaster University, 1200 Main Street West, Hamilton, Ont. L8N 3Z5, Canada. changp@fhs.mcmaster.ca
SOURCE: Artificial Cells, Blood Substitutes, and Immobilization Biotechnology, (2000) 28/1 (1-24).
Refs: 78
ISSN: 1073-1199 CODEN: ABSBE4
COUNTRY: United States
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 022 Human Genetics
025 Hematology
LANGUAGE: English

SUMMARY LANGUAGE: English

AB Hemophilia A and B are X-linked genetic disorders caused by deficiency of the coagulation factors VIII and IX, respectively. Because of the health hazards and costs of current product replacement therapy, much effort is devoted to the development of gene therapy for these disorders. Approaches to gene therapy for the hemophilias include: **ex vivo gene therapy** in which cells from the intended recipients are explanted, genetically modified to secrete Factor VIII or IX, and reimplanted into the donor; **in vivo gene therapy** in which Factor VIII or IX encoding vectors are directly injected into the recipient; and non-autologous gene therapy in which universal cell lines engineered to secrete Factor VIII or IX are enclosed in immuno-protective devices before implantation into recipients. Research into these approaches is aided by the many murine and canine models available. While **problems** of achieving high and sustained levels of factor delivery, and issues related to efficacy, safety and cost are still to be resolved, progress in gene therapy for the hemophilias has been encouraging and is likely to reach human clinical trial in the foreseeable future.

L9 ANSWER 28 OF 33 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN

ACCESSION NUMBER: 1998409154 EMBASE

TITLE: Long-term amelioration of bilirubin glucuronidation defect in Gunn rats by transplanting genetically modified immortalized autologous hepatocytes.

AUTHOR: Tada K.; Roy-Chowdhury N.; Prasad V.; Kim B.-H.; Manchikalapudi P.; Fox I.J.; Van Duijvendijk P.; Bosma P.J.; Roy-Chowdhury J.

CORPORATE SOURCE: Dr. J. Roy-Chowdhury, Marion Bessin Liver Research Center, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10462, United States

SOURCE: Cell Transplantation, (1998) 7/6 (607-616).
Refs: 24

PUBLISHER IDENT.: ISSN: 0963-6897 CODEN: CTRAES
S 0963-6897(98)00035-9

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 009 Surgery
022 Human Genetics
048 Gastroenterology

LANGUAGE: English

SUMMARY LANGUAGE: English

AB **Ex vivo gene therapy**, in which hepatocytes are harvested from mutants, retrovirally transduced with a normal gene and transplanted back into the donor, has been used for correction of inherited metabolic defects of liver. Major drawbacks of this method include limited availability of autologous hepatocytes, inefficient retroviral transduction of primary hepatocytes, and the limited number of hepatocytes that can be transplanted safely. To obviate these **problems**, we transduced primary hepatocytes derived from inbred bilirubin-UDP-glucuronosyltransferase (UGT)-deficient Gunn rats by infection with a recombinant retrovirus expressing temperature-sensitive mutant SV40 large T antigen ((ts)T). The immortalized cells were then transduced with a second recombinant retrovirus expressing human B-UGT, and a clone expressing high levels of the enzyme was expanded by culturing at permissive temperature (33.5°C). At 37.5°C, (ts)T antigen was degraded and the cells expressed UGT activity toward bilirubin at a level approximately twice that present in normal rat liver homogenates. For seeding the cells into the liver bed, 1 x 10⁷ cells were injected into the spleens of syngeneic Gunn rats five times at 10-day intervals. Excretion of bilirubin glucuronides in bile was demonstrated by HPLC analysis and serum bilirubin levels were reduced by 27 to 52% in 40 days after the first transplantation and remained so throughout the duration of the study (120 days). None of the transplanted Gunn rats or SCID mice transplanted

with the immortalized cells developed tumors.

L9 ANSWER 29 OF 33 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 97014586 EMBASE
DOCUMENT NUMBER: 1997014586
TITLE: Vectors - shuttle vehicles for gene therapy.
AUTHOR: Wilson J.M.
CORPORATE SOURCE: J.M. Wilson, Institute for Human Gene Therapy, 204 Wistar, 3601 Spruce Street, Philadelphia, PA 19104-4268, United States
SOURCE: Clinical and Experimental Immunology, Supplement, (1997) 107/1 (31-32).
Refs: 4
ISSN: 0964-2536 CODEN: CEISEE
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 022 Human Genetics
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Gene therapy is being considered for the treatment of various inherited and acquired disorders. The basic premise of this new therapeutic modality is manipulation of gene expression towards a therapeutic end. The early development of the field focused on a technique called **ex vivo gene therapy** in which autologous cells are genetically manipulated in culture prior to transplantation. Recent advances have stimulated the development of in vivo gene therapy approaches based on direct delivery of the therapeutic gene to cells in vivo. The rate-limiting technologies of gene therapy are the gene delivery vehicles, called vectors, used to accomplish gene transfer. The most efficient vectors are based on recombinant versions of viruses with retroviral vectors serving as prototypes. This viral vector system has been exploited in ex vivo approaches of gene therapy in which cultured, dividing cells are transduced with the recombinant virus resulting in integration of the proviral DNA into the chromosomal DNA of the recipient cell. The use of retroviral vectors in gene therapy has been restricted to ex vivo approaches because of **difficulties** in purifying the virion and the requirement that the target cell is dividing at the time of transduction. More recently, vectors based on adenoviruses have been developed for in vivo gene therapy. These viruses can be grown in large quantities and highly purified. Importantly, they efficiently transduce the recombinant genome into non-dividing cells. Applications include in vivo gene delivery to a variety of targets such as muscle, lung, liver and the central nervous system. Clinical trials of in vivo delivery with adenoviruses have been undertaken for the treatment of cystic fibrosis.

L9 ANSWER 30 OF 33 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 96339142 EMBASE
DOCUMENT NUMBER: 1996339142
TITLE: Adenovirus for neurodegenerative diseases: In vivo strategies and **ex vivo gene therapy** using human neural progenitors.
AUTHOR: Sabate O.; Barkats M.; Buc-Caron M.-H.; Castel-Barthe M.-N.; Finiels F.; Horellou P.; Revah F.; Mallet J.
CORPORATE SOURCE: CNRS C 9923, LGMNPD (LGN), Hopital de la Pitie, 83 Boulevard de l'Hopital, 75013 Paris, France
SOURCE: Clinical Neuroscience, (1995) 3/5 (317-321).
ISSN: 1065-6766 CODEN: CINUE5
COUNTRY: United States
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 008 Neurology and Neurosurgery
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The discovery of major neurodegenerative mechanisms has opened the way to

the development of novel therapeutic approaches. Gene therapy now enables researchers to overcome certain **problems** inherent to pharmacotherapy and to the grafting of embryonic cells. The production of recombinant adenoviruses are promising for *in vivo* gene therapy involving neuroprotective (Ad-SOD), neurotrophic (Ad-NGF) as well as restorative (Ad-TH) strategies. In addition, human neural progenitors offer great potential as vehicles for **ex vivo gene therapy** to replace degenerated cells in advanced stages of neurodegenerative diseases. This paper describes the clinical value of the new generations of adenoviral vectors.

L9 ANSWER 31 OF 33 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 96036484 EMBASE
DOCUMENT NUMBER: 1996036484
TITLE: Toward development of an implantable tissue engineered liver.
AUTHOR: Davis M.W.; Vacanti J.P.
CORPORATE SOURCE: Department of Surgery, Harvard Medical School, 300 Longwood Avenue, Boston, MA 02115, United States
SOURCE: Biomaterials, (1996) 17/3 (365-372).
ISSN: 0142-9612 CODEN: BIMADU
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 027 Biophysics, Bioengineering and Medical Instrumentation
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Hepatocyte transplantation on implantable devices is a tissue engineering approach to improve the treatment of liver disease and the efficacy of **ex vivo gene therapy**. Diverse physiological functions and high metabolic activity of the liver represent significant **challenges** to engineering implantable devices that provide long-term hepatic support. Liver tissue engineering research has explored alternatives to direct hepatocyte injection that include hepatocyte attachment to microcarriers, encapsulation and transplantation on biodegradable polymer scaffolds. Successful function of hepatocytes transplanted on implantable devices in animal models has been documented by production of albumin and other liver-specific markers, and clearance of bilirubin and urea metabolites. Strategies used to achieve these successes are reviewed, with particular emphasis on biodegradable polymer scaffolds, and two areas of investigation that may improve the function of implantable tissue engineered liver devices are highlighted.

L9 ANSWER 32 OF 33 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 95280987 EMBASE
DOCUMENT NUMBER: 1995280987
TITLE: Curing IDDM - prevention, repair or replacement.
AUTHOR: Bone A.J.; Cole D.R.
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SOURCE: Endocrinology and Metabolism, (1995) 2/3 (169-184).
ISSN: 1074-939X CODEN: ENDMEM
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 003 Endocrinology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Much of our knowledge about the nature of the disease processes operating in IDDM stems from studies performed in animal models. In particular, the diabetic syndromes of both the NOD mouse and BB rat have been shown to share a number of similarities - genetic, immunological, hormonal, environmental - with that of human IDDM. Thus, specific genetic lesions,

pathogenic mechanisms and environmental influences have been described in the animal models of IDDM which appear to exist, at least in part, in patients with diabetes. It is the detailed understanding of how these factors interact to produce targeted beta-cell destruction that will lead the way to the discovery of new therapies for the prevention and treatment of IDDM. The animal models, with their prolonged prediabetic period, provide us with the opportunity to assess therapeutic intervention strategies aimed at preventing development of IDDM, but we must be aware of the potential pitfalls of overinterpreting findings from animal studies and circumspect when relating them directly to the human disease. It would however, be equally foolish to ignore important animal data indicating possible shortcomings of therapies destined for evaluation in human clinical trials. In this context, it is difficult to accept the rationale behind the use of generalized immunosuppressants, like cyclosporin (CSA), to treat patients with established, albeit recently diagnosed, diabetes. Early animal studies provided encouraging data suggesting a delay in diabetes onset in susceptible individuals treated prophylactically with CSA but there was no indication of any benefit in overtly diabetic animals. Furthermore, the effects of CSA were not sustained following the cessation of therapy. Thus, the findings of, at best, transient improvements in insulin requirement in newly diagnosed patients given CSA were predictable and as such should have been weighed up against the risk of using a drug with known cytotoxic effects. The basic concept of restricting the use of 'protective' treatments to situations where there is still something to protect applies equally well to therapies aimed at modulating islet cell defence and repair mechanisms. Such approaches do however, have the advantage of being specific for the target organ with a subsequently reduced risk of side-effects. It is apparent however that even using targeted therapy the best results will be achieved when treatment is administered at the earliest stages of the disease. Thus, the advances in therapeutic intervention in IDDM will have to be paralleled by similar progress in the early identification of individuals at risk if prevention of diabetes is to become a reality. Failure to prevent the onset of overt IDDM only leaves the option of insulin replacement therapy. There are major technological hurdles to be overcome in the development of a safe and reliable artificial endocrine pancreas. Pancreatic transplantation would seem to offer the advantages of improved, more physiological metabolic control but, even if morbidity were reduced, the **problems** associated with organ supply make this approach of limited benefit. The advances in automated islet isolation techniques have opened the way to transplantation procedures using purified islet tissue. This approach permits graft manipulation to reduce immunogenicity prior to implantation, a very straightforward procedure compared to whole organ grafting, but still the **problems** associated with tissue supply remain. The supply of human donor pancreata will never be adequate to cope with demand and therefore other sources of tissue are being explored. Xenograft protection technology has been attempted but even with the use of hybrid bioartificial organs function has only been shown with the use of generalized immunosuppression. However, using human islets progress in membrane technology is encouraging and studies in non-immunosuppressed patients are awaited. Very recently, **ex vivo gene therapy** has been used to create glucose-sensitive, insulin secreting cell lines which potentially offer an alternative solution to overcoming the limitations of insufficient material for transplantation. Finally, as we move towards the 21st century, we would urge those who seek a cure for IDDM to heed the words of Publius Syrus spoken some 2000 years ago: 'Better use medicine at the outset than at the last moment'.

AUTHOR: herpes simplex virus-1 vector.
Lu B.; Gupta S.; Federoff H.
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SOURCE: Hepatology, (1995) 21/3 (752-759).
ISSN: 0270-9139 CODEN: HPTLD
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
022 Human Genetics
048 Gastroenterology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB A defective amplicon herpes simplex virus-1 (HSV-1) vector, HSVlac, was used to transfer an E. cell lacZ reporter gene into primary hepatocytes. The lacZ gene was driven by the HSV immediate early (IE) 4/5 promoter. Use of the HSVlac vector resulted in highly efficient gene transfer. Because **difficulties** in culturing primary hepatocytes impose limitations in **ex vivo gene therapy**, we sought to determine whether use of the HSVlac vector could simplify gene transfer. Therefore, we incubated HSVlac with primary hepatocytes in suspension and found that the lacZ gene was still transferred with great rapidity and efficiency. To examine lacZ expression in transduced hepatocytes *in vivo*, we used a mouse hepatocyte transplantation system. In congenic recipients of primary hepatocytes transduced with HSVlac in suspension, the lacZ gene was expressed in liver and spleen up to 2 weeks. However, survival of transplanted hepatocytes, as well as persistence of HSVlac genome in recipient organs, was demonstrated for up to an 11-week duration of the experiment. These findings suggest that *in vivo* regulation of the HSV IE4/5 promoter was responsible for the short-term expression of lacZ, which should be overcome by the use of liver-specific promoters. Therefore, our results indicate the feasibility of hepatic gene transfer with a defective HSV-1 vector.

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